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THE SOLUBILITY OF GLIADIN

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The solubility of gliadin in different solvents has never been investigated thoroly. Various researches have been carried out on certain phases of the question, but in no case has the work been extensive.

In Acids

It is well known that gliadin is readily soluble in dilute aqueous solutions of strong alkalies, but its solubility in dilute aqueous solutions of acids has not been investigated fully. Accordingly it was thought advisable to undertake this work, using different acids of varying concentrations.

First a sample of gliadin was prepared from wheat flour by the method of Osborne.[1] Its solubility in different acids of varying concentrations was determined by the following method: A measured volume of the acid under investigation was placed in a suitable bottle, and one gram of gliadin in a fine powder was added for each 100 cc. of the solvent. A preliminary investigation had shown that this amount of gliadin was a sufficient excess for all concentrations of acids used. As a preservative, five drops of toluene was always added for each 100 cc. of mixture. The bottle was corked tight, and shaken continuously on a shaking machine for eight hours. The mixture was allowed to stand over night, then filtered, and nitrogen was determined in 100 cc. of the filtrate by the Kjeldahl-Gunning method. The nitrogen was computed to gliadin by use of the factor 5.70. The results are set forth in Table I. In this table as well as in all the following tables the solubility of gliadin is expected in milligrams per 100 cc.

[1] Osborne, Thomas B., Proteins of the Wheat Kernel, Carnegie Institute, Washington, D. C. 1907.

TABLE I
SOLUBILITY OF GLIADIN IN ACIDS
(Entire time, 24 hrs.; temp. 25° C.; charge 1 gm. per 100 cc.)

Acid	Concentration					
	n/1	n/10	n/100	n/1000	n/10,000	n/100,000
	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.
HCl	55.0	66.1	139.7	135.7	96.6	70.0
H_2SO_4	63.2	74.1	120.8	126.5	89.0	71.6
CH_3COOH	173.3	165.4	130.9	111.7	81.6	76.1

Proteins are very susceptible to change, consequently the method of preparation and purification has a great deal to do with the physical and chemical characteristics of each individual of this class. Therefore the next point investigated was the influence of the method of preparation and purification on the solubility of gliadin. In this investigation three different samples of gliadin were used. These samples were made and purified as follows:

Sample No. 1.—This sample was prepared by extracting well washed gluten with cold 70 per cent ethyl alcohol. The undissolved glutenin was filtered off and the alcoholic filtrate evaporated to a syrup under reduced pressure. The temperature was not allowed to go above 65° C. The syrup was cooled and then poured, with constant stirring, into a 100-fold volume of ice-cold distilled water containing ten grams of sodium chloride per liter. The precipitated gliadin was washed with water three times by decantation, redissolved in 70% ethyl alcohol, again filtered, and evaporated to a syrup as before, then cooled and poured into water containing sodium chloride. This purification was repeated four times altogether. The syrup from the final evaporation was poured into 100 volumes of cold absolute ethyl alcohol. The precipitated gliadin was filtered off, washed with absolute ethyl alcohol, then with ether, and finally dried in a desiccator over sulphuric acid.

Sample No. 2.—This sample was prepared in exactly the same way as Sample No. 1, except that methyl alcohol was used throughout instead of ethyl alcohol.

Sample No. 3.—This sample was prepared in the same way as No. 1, except that after the final evaporation of the alcoholic filtrate, the syrup was poured into hot absolute ethyl alcohol instead of into cold absolute ethyl alcohol.

Hydrochloric acid is the most representative of the acids used, all things being considered, because of the high degree of ionization of its aqueous solutions, as well as its extensive use in protein research, altho gliadin is more soluble in acetic acid of equivalent concentrations. Hence hydrochloric acid was used as solvent in this investi-

gation. Nitrogen was determined in the same way as described in the preceding experiment. The results are set forth in Table II.

TABLE II

INFLUENCE OF METHOD OF PREPARATION ON THE SOLUBILITY OF GLIADIN IN HYDROCHLORIC ACID SOLUTIONS

(Time, 24 hours; temp. 25° C.; charge, 1 gm. per 100 cc.)

Concentration of HCl	Sample No. 1	Sample No. 2	Sample No. 3
	Mgms.	Mgms.	Mgms.
n/1	49.6	55.0	44.2
n/10	58.4	60.1	61.3
n/100	355.4	129.7	242.8
n/1000	354.8	125.4	197.2
n/10,000	169.9	108.3	45.3
n/100,000	92.3	93.8	26.2

At this point the question arose regarding the effect of drying on the solubility of gliadin. From the behavior of other proteins it was inferred that moist gliadin was more soluble in a given solvent than the dry. To test out this point a sample of gliadin was made by method No. 1 with the exception that instead of pouring the syrup from the final evaporation under reduced pressure into cold absolute alcohol, it was poured, with constant stirring, into cold distilled water containing 10 grams of sodium chloride per liter. The resulting precipitate of gliadin was divided into two parts. One part was dried by allowing it to stand in 100 times its own weight of absolute ethyl alcohol over night, then washing several times with a fresh portion of absolute alcohol, then with ether, and finally completing the drying in a desiccator over sulphuric acid. The second portion was washed thoroly with distilled water, then filtered, and the moist precipitate dried somewhat between filter paper. The moisture was determined on a weighed portion by drying in an air oven at 100° C. to constant weight. During the moisture determination the remainder of the moist gliadin was kept in a tight container and preserved with a few drops of toluene. As soon as the total moisture was known, the solubility of the moist gliadin was determined by quickly weighing a portion to 0.1 gram and adding the solvent in such an amount that the equivalent of one gram of dry gliadin was present for each 100 cc. of solvent.

Finally the solubility of the dried gliadin was determined using one gram for each 100 cc. of solvent. Water was the solvent used in this series, with different periods of time as indicated in Table III.

TABLE III

SOLUBILITY OF DRY AND MOIST GLIADIN IN WATER

(Temp. 25° C; charge, 1 gm. per 100 cc.)

Time, hrs.	24	48	72	96	120	144	168	196	240
	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.
Dry	53.0	60.4	63.0	65.6	69.0	73.1	75.6	78.7	79.8
Moist	73.0	105.2	119.3	120.2	133.6	142.8	151.9	162.7	163.4

Throughout the entire preliminary investigation it was noticed that the amount of the excess of gliadin used had a great influence on the amount dissolved in a given solvent. The preliminary results showed that the solubility was approximately proportional to the excess of the solute present. This, of course, would indicate that the solutions were not saturated. Therefore, an experiment was carried out in order to test this point.

The concentrations of the solute were varied between 0.1 and 5.0 grams per 100 cc. of the solvent. In every instance some undissolved solute was present at the end of the specified time.

The results are set forth in Table IV.

TABLE IV

INFLUENCE OF AMOUNT OF SOLUTE ON SOLUBILITY

(Temp. 25° C.; time, 24 hours)

Solvent		Concentration of gliadin per 100 cc. of solution					
		0.1 gm.	0.25 gm.	0.5 gm.	1.0 gm.	2.5 gm.	5.0 gm.
	Concentration	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.
H_2SO_4	n/10	12.0	23.4	41.9	74.1	119.1	175.8
"	n/100	16.4	31.9	61.0	120.8	291.3	536.4
"	n/1000	17.7	39.3	67.8	126.5	268.2	405.3
HCl	n/10	12.5	22.2	35.3	57.0	107.2	157.3
"	n/100	63.0	91.1	125.4	200.3	439.1	838.6
"	n/1000	46.2	57.8	118.1	148.6	348.2	661.5
CH_3COOH	n/10	57.2	91.8	148.7	165.9	399.8	695.4
H_2O	.....	8.6	18.2	42.8	95.2	178.1	266.2

The results as shown in Table IV suggested the following investigation regarding the time factor on the solubility, i. e., the length of time necessary to produce saturation. Owing to coagulation effects, the application of heat was out of the question; hence the time had to be extended and the temperature kept at 25° C. The length of time, the different acids used and their concentrations, respectively, are given in the table of results. As heretofore, the quantities dissolved are expressed in milligrams of gliadin dissolved in 100 cc. of the solvent.

TABLE V

INFLUENCE OF TIME ON SOLUBILITY

(Temp. 25° C.; charge, 1 gm. per 100 cc.)

Reagent	Concentration	Time, hours									
		24	48	72	96	120	144	168	192	216	240
		Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.	Mgms.
H_2SO_4	n/10	63.8	72.4	74.4	74.7	76.8	77.5	78.0	78.8	80.0	82.1
"	n/100	127.1	141.7	151.6	154.5	156.8	157.9	160.2	167.9	168.1	169.0
"	n/1000	106.1	108.6	112.0	114.6	118.0	119.9	120.6	121.1	122.3	124.1
HCl	n/10	22.3	28.5	31.9	33.9	34.2	34.5	36.2	36.5	38.0	39.9
"	n/100	86.1	93.2	95.8	96.6	98.1	98.0	100.4	100.5	102.0	105.5
"	n/1000	40.8	48.0	50.1	52.3	57.4	65.0	70.4	75.3	76.1	78.2
CH_3COOH	n/10	47.6	48.5	48.7	49.0	49.0	49.6	49.6	50.1	51.0	51.2
NaCl	n/10	23.7	27.1	27.9	27.9	28.5	29.6	32.5	34.2	36.2	36.2
"	n/100	12.0	14.0	16.5	16.8	18.2	23.0	28.2	34.1	39.6	73.5
CH_3OH	10%	72.1	77.5	79.8	85.8	96.0	97.5	99.0	105.5	106.1	115.1
"	20%	77.8	80.7	82.4	85.7	99.2	111.2	117.1	128.3	141.6	144.9
"	30%	65.0	73.2	76.7	78.7	79.2	80.4	83.4	93.8	94.1	97.5
"	40%	68.4	69.8	66.7	70.1	71.2	72.8	73.0	73.9	74.6	75.0
"	50%	82.7	85.2	84.6	85.2	85.5	85.9	86.1	86.3	87.0	87.2
"	60%	189.5	203.2	207.8	212.9	214.6	218.5	218.9	219.4	220.0	221.2

TABLE VI

SOLUBILITY OF GLIADIN IN SOLUTIONS OF KNOWN pH

(Time, 24 hours; Temp. 25° C.; charge, 1.0 gm. per 100 cc.)

pH	0.00	1.01	2.08	3.31	4.24	6.14	6.78	.....	.....	.....	.....	.....
HCl												
Mgm. gliadin	55.00	60.10	129.70	125.40	108.30	93.80	66.50	.....	.....	.....	.....	.....
pH	7.08	7.93	8.74	9.72	12.16	13.07	.....	.....	.....	.....	.....	.....
NaOH												
Mgm gliadin	45.60	130.00	132.20	150.50	189.00	202.00	.....	.....	.....	.....	.....	.....
pH	2.35	3.97	4.85	5.06	5.53	5.87	6.54	6.85	7.56	8.03	8.94	10.26
Phosphates												
Mgm. gliadin	60.40	70.40	35.00	29.60	18.50	15.00	10.50	11.40	17.40	20.00	26.00	39.50

Throughout all the foregoing investigations with acids it was noticed that the hydrogen-ion concentration of the solvent was an important factor in the solubility of gliadin. This fact called for further investigation. Accordingly three series of experiments were planned in which the solubility of gliadin was determined in solutions of known hydrogen-ion concentration. In the first series hydrochloric acid of varying concentrations was used as solvent; in the second sodium hydroxide, and in the third a mixture of the primary and secondary potassium phosphates together with either phosphoric acid or sodium hydroxide.

Solutions of the above substances of varying concentrations were made up, and the hydrogen-ion concentration of each was accurately determined with the hydrogen electrode. These different solutions were then used as solvents for gliadin just as in the preceding experiments. With the phosphate series the different solutions were always 0.01 molar with reference to the respective salts of their mixtures. To obtain solutions of higher H-ion concentration than that given by 0.01 molar mono-potassium phosphate, varying amounts of phosphoric acid were added to 0.01 molar solutions of the former. With lower H-ion concentrations than 0.01 molar di-potassium phosphate, varying amounts of sodium hydroxide solution were added to it.

In both the hydrochloric acid series and the sodium hydroxide series a one-gram charge per 100 cc. of solvent was used. In the phosphate series the charge was 0.1 gram for each 100 cc. of solvent.

Table VI contains the results.

In Alkalies

The solubility of gliadin in aqueous solutions of sodium hydroxide can be seen in Table VI. Other investigators have found that gliadin is readily soluble in aqueous solutions of other alkalies and alkaline earths. Carbon dioxide will precipitate gliadin from aqueous solutions of sodium hydroxide or sodium bicarbonate. Hence it was thought advisable to investigate the solubility of gliadin in aqueous solutions of sodium carbonate.

The method of procedure was exactly the same as in the previous experiments. As gliadin is so very slightly soluble in solutions of the normal carbonates, A 0.1 gram charge was used.

Table VII contains these results.

TABLE VII

SOLUBILITY OF GLIADIN IN SODIUM CARBONATE SOLUTIONS

(Time, 24 hours; temp. 25° C.; charge, 0.1 gm. per 100 cc.)

Concentration	Gliadin	Concentration	Gliadin	Concentration	Gliadin	Concentration	Gliadin
	Mgms.		Mgms.		Mgms.		Mgms.
Saturated	2.9	0.09 m	7.6	0.02 m	10.0	0.004 m	8.6
0.6 m	2.9	0.08 m	7.7	0.01 m	10.5	0.003 m	8.0
0.5 m	3.4	0.07 m	8.0	0.009 m	12.0	0.001 m	7.2
0.4 m	3.7	0.06 m	8.5	0.008 m	10.5	0.0005 m	4.9
0.3 m	4.2	0.05 m	8.7	0.007 m	10.1	0.0001 m	3.9
0.2 m	5.1	0.04 m	8.9	0.006 m	9.5	.....	...
0.1 m	7.4	0.03 m	9.2	0.005 m	9.1	.....	...

In Aqueous Solutions of Neutral Salts and in Aqueous Methyl Alcohol Solutions

It has long been known that gliadin from wheat is practically insoluble in aqueous solutions of neutral salts. Sodium chloride, being a characteristic neutral salt, has been used almost exclusively in all such investigations. In the ordinary method of preparing gliadin the alcoholic extract from the gluten is evaporated to a syrup and poured into a large volume of ice-cold distilled water containing ten grams of sodium chloride per liter. The gliadin, being less soluble in sodium chloride solutions than in water alone, is precipitated.

During some investigations on the gluten proteins of flour, it was noticed that the gliadin was much more soluble in the sodium chloride solutions than in water alone. The sodium chloride used was the commercial grade and consequently contained other salts. It was found to contain considerable magnesium chloride as well as sulphates of magnesium, sodium, and potassium.

Data in the literature bearing on the solubility of gliadin in the aqueous solutions of these salts are limited. Bailey and Blish (1915) indicated an appreciable solubility of gliadin in dilute aqueous solutions of sodium chloride. It appeared desirable to expand this study to include other saline solutions and consequently the solubility of gliadin in aqueous solutions of the more common neutral salts was investigated in some detail.

The procedure was the same as in the previous experiments, and the resulting data are recorded in Table VIII.

The solubility of gliadin in ethyl alcohol has been investigated by others. It was found that the maximum solubility in ethyl alcohol-water mixtures occurred at a concentration of 60 to 70% alcohol by volume. It was found also that gliadin was practically insoluble in absolute ethyl alcohol.

TABLE VIII

SOLUBILITY OF GLIADIN IN AQUEOUS SOLUTIONS OF NEUTRAL SALTS
(Time, 24 hours; temp. 25° C.; charge, 0.1 gm. per 100 cc.)

Concentration	Salts								
	NaCl	KCl	$CaCl_2$	$BaCl_2$	$SrCl_2$	$MgCl_2$	Na_2SO_4	K_2SO_4	$MgSO_4$
Saturated	2.7	1.1	1.1	1.2	...	84.4	4.0	2.2	0.0
1.0 Molar	1.1	1.4	0.7	0.4	0.6	59.2	...	2.1	1.1
0.5 "	1.7	1.5	0.7	0.8	0.8	28.5	...	2.2	1.1
0.1 "	1.8	1.7	0.9	0.9	0.8	14.5	8.0	2.0	1.7
0.05 "	2.0	1.7	1.0	1.1	0.9	5.7	11.4	...	2.3
0.01 "	2.1	1.9	1.4	1.3	1.2	5.4	9.1	...	2.3
0.005 "	2.4	2.0	1.5	1.3	1.4	5.0	7.4	...	2.4
0.001 "	2.4	2.1	1.8	1.7	1.5	4.0	4.0	...	2.5
0.0005 "	2.7	2.5	2.0	1.9	1.7	3.1	3.4	...	2.7
0.0001 "	2.8	2.7	2.4	2.5	2.4	3.0	2.9	...	2.9

The next point to be taken up was the solubility of gliadin in aqueous solutions of methyl alcohol. Chemically pure methyl alcohol was used. The procedure was the same as in the preceding experiments. The results are to be found in Table IX.

TABLE IX

SOLUBILITY OF GLIADIN IN AQUEOUS SOLUTIONS OF METHYL ALCOHOL
(Time, 24 hours; temp. 25° C.; charge, 1 gm. per 100 cc.)

CH_3OH, % by volume	10	20	30	40	50	60	70	80	90	100
Gliadin, mgms.	101.2	104.0	106.0	116.6	126.5	133.1	132.2	114.0	28.5	8.6

Discussion of Results

Table I.—The results in this table show that acetic acid is the best solvent for gliadin; also that the best concentration for the maximum solubility is about n/100 for hydrochloric and sulfuric acids. This concentration represents a pH value of 2 to 3. In the case of acetic acid the maximum solubility occurs at a concentration of about 1.0 N. This corresponds to a pH value of about 2.3.

With concentrations greater than pH 2.0 the solubility falls off very rapidly. With concentrations less than pH 2.0 the solubility decreases more slowly.

Table II.—The results in this table confirm the results in Table I with regard to the optimum concentration of acids (about pH 2.0) for the maximum solubility of gliadin. All the different preparations of gliadin show their maximum solubility at this same point.

In addition to the above, the results show that ethyl alcohol is the best solvent to use in the preparation of gliadin; also that hot absolute alcohol decreases the solubility of gliadin very markedly.

Table III.—The results here show that moist gliadin is much more soluble in water than dry gliadin. They show also that solu-

bility decreases with time. Another point to notice is that it takes a long time to obtain a saturated solution of gliadin.

Table IV.—The results in this table show very clearly the influence of the amount of solute on the solubility. With concentrations of acids in which gliadin shows the maximum solubility (n/100-n/1000), the solubility is directly proportional to the concentration of the solute. This indicates that the saturation point had not been reached in 24 hours even with a charge of five grams. The curves for different acids of equivalent concentrations are very similar, indicating that solubility depends to a great extent on the H-ion concentration.

Table V.—The results in this table show that the amount of gliadin dissolved increases very slowly after 72 hours. The differences in solubility in this table and those with the same acids and same concentrations in the preceding tables are due to the fact that different samples of gliadin were used. It has been shown that the method of preparation has considerable influence on the solubility.

Table VI.—The influence of the hydrogen-ion concentration on the solubility of gliadin can readily be seen by an inspection of the results in this table. The maximum solubility in acids or acid salts occurs at pH 2 to 3; at greater concentrations the solubility falls off very rapidly. As the pH values approach the neutral point, the solubility decreases more slowly. The minimum solubility occurs at about pH 6.5 according to the phosphate series. It must be remembered that with very dilute solutions of both hydrochloric acid and sodium hydroxide (greater than 1/1000 N) the determination of the H-ion concentration with the hydrogen electrode is not so accurate, owing to the lack of buffer action. On the alkaline side of the neutral point the solubility increases more rapidly than on the acid side.

Table VII.—Gliadin is slightly soluble in sodium carbonate solutions, the maximum occurring at about 0.009 molar.

Table VIII.—The solubility of gliadin in aqueous solutions of neutral salts is very slight and for the most part very similar. As the concentrations of the salts decreased, the solubility of gliadin approached that for gliadin in distilled water which was found to be 2.9 mgms. per 100 cc. for a 24-hour period at 25° C. Unexpected results were obtained with $MgCl_2$. Gliadin is quite soluble in solutions of this salt, which fact explains the abnormal results mentioned above.

Table IX.—Gliadin is quite soluble in aqueous methyl alcohol solutions. The maximum solubility occurs at a concentration of from 60 to 70 per cent of methyl alcohol by volume.

Summary

Acetic acid is the best solvent for gliadin. In the case of hydrochloric and sulfuric acids the optimum concentration for the maximum solubility lies between 1/100 and 1/1000 N., and with all three acids the optimum pH is about 2.0. The minimum solubility occurs at about pH 6.5. Another minimum point is found at about pH 0.0.

The method of preparation has considerable influence on the solubility of gliadin. Methyl alcohol and hot ethyl alcohol should be avoided.

Drying, even at low temperatures, decreases the solubility.

At least 72 hours are required for the saturation point to be reached.

Gliadin is very soluble in sodium hydroxide solutions, but very slightly soluble in solutions of sodium carbonate.

As a rule gliadin is very slightly soluble in aqueous solutions of neutral salts. The reverse, however, is true of magnesium chloride.

Aqueous methyl alcohol solutions are good solvents for gliadin, the maximum solubility occurring at a concentration of 60 to 70 per cent of methyl alcohol by volume.

THE INDIVIDUALITY OF GLUTENIN[1]

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Woodman (1922) studied certain optical properties of gluten proteins prepared from a strong Canadian wheat flour and a week English flour, respectively. He concluded that his two gliadin preparations were identical, but that the two glutenins differed in the configurations of their respective molecules. The latter conclusion was based upon his finding that the glutenins showed appreciable differences in specific rotatory power, as well as in their racemization rates when they were permitted to stand for a considerable period of time in alkaline (sodium hydroxide) solution at 37° C. He expressed the opinion that such variations in the chemical configurations of different glutenins are probably responsible for variations in flour strength. This deduction, if true, is of great importance to the wheat and flour industry, but the

[1] Published with the approval of the Director as Paper No. 10, Journal Series, Nebraska Agricultural Experiment Station.

fact that it was based upon evidence from but *two* preparations of glutenin made it less convincing than it would have been had more samples been studied. Further cause for uncertainty arises from the fact that the standard method for preparing and purifying glutenin involves frequent prolonged standing in dilute alkaline solution.

For these, as well as other reasons, Blish and Pinckney (1924) conducted a similar study with *seven* carefully purified glutenin preparations from flours of widely different sources and baking qualities. Three of these glutenins were prepared in this laboratory. Four were prepared in another and far-distant laboratory, quite independently of the other three. Six of the seven samples were identical, and the seventh varied but slightly. It was therefore concluded that "it is highly improbable that variations in the respective flour strengths of our commercially important wheats can ever be attributed to differences in the chemical configurations of their respective glutenin molecules."

Quite recently Halton (1924) has announced the separation of a single glutenin preparation into two fractions. He dissolved a sample of purified glutenin in sodium hydroxide, and slowly added 1N HCl until he obtained a precipitate which completely settled to the bottom of the container, using the minimum amount of HCl. He then drew off the supernatant liquid, added more HCl, drop by drop, and obtained a second precipitate which settled completely after 3.8 cc. HCl per liter of solution had been added. He did not state the respective hydrogen-ion concentrations at which the two precipitates were obtained. He found, however, that they had slightly different initial specific rotations when dissolved in alkali. When the alkaline solutions were allowed to stand at 37°, and specific rotations were plotted against definite time intervals, the racemization curves were practically parallel, but not coincident. From this observation he states "Glutenin is not a single protein, but consists of at least two fractions differing in their optical rotation * * * glutenin from a strong wheat flour has a higher optical rotation than that from a weak flour. A strong wheat would therefore be expected to contain a greater percentage of the fraction with the high optical rotation."

Experimental

As the method of preparing and purifying glutenin involves several operations, in each of which the glutenin must be dissolved in dilute alkali, precipitated at one definite hydrogen-ion concentration and allowed to settle, while the supernatant liquid is discarded, it is difficult to understand how Halton was able to retain more than one

glutenin fraction during the entire process of purification even on the assumption that there was more than one present in the original flour.

Before accepting Halton's conclusions, it was considered advisable to try his procedure with several glutenin preparations available from the work of Blish and Pinckney (1924). Therefore portions of five different preparations of glutenin were dissolved in dilute sodium hydroxide. Each solution was treated with dilute hydrochloric acid according to Halton's procedure. As soon as the first cloudiness was observed, the acid was added a drop at a time, shaking vigorously after each drop, and allowing a few minutes for possible settling. In each case scrupulous care was taken to use only the minimum amount of acid necessary to produce a clear separation of precipitate from supernatant liquid. In no instance could more than one fraction be obtained. In all five cases the glutenin was completely precipitated in one fraction, for no protein remained in any of the supernatant liquids, as was shown by testing a portion of each with phosphotungstic acid solution.

Realizing that more than one glutenin fraction might have been present in the flours from which the glutenins were originally prepared, but that all but one fraction had possibly been lost during the purification process, recourse was had to the principle involved in Blish and Sandstedt's (1925) new method for the preparation and direct quantitative determination of glutenin. By this procedure *all* of the flour protein may be brought into solution in 60-70% methyl alcohol which is but slightly alkaline with NaOH. From this solution glutenin alone may be quantitatively precipitated by the addition of an exact small amount of HCl. Extracts from three flours were prepared in this way. Glutenin from each extract was precipitated with the least quantity of HCl which would give sharp precipitation. In no case could a second fraction be found in the supernatant liquid from the first precipitation.

It appeared desirable to account, if possible, for the glutenin "fractions" obtained by Halton (1924). Gortner[2] suggested that possibly Halton's glutenin had become slightly racemized before he fractionated it, and that racemized protein may have an isoelectric point differing somewhat from that of the natural protein. When this idea was applied to slightly racemized glutenin, such was found to be the case. A one per cent solution of glutenin in .5N NaOH was allowed to stand for about 20 hours at 37 degrees. One hundred cc. of this solution was then added to 100 cc. of a freshly prepared 1% solution

[2] Private communication from Dr. R. A. Gortner of the University of Minnesota.

of another portion of the same glutenin in .5N NaOH. To this solution, containing both the "natural" and slightly racemized protein, 1N HCl was added as before until a definite precipitate settled to the bottom of the flask, using the least possible acid. The settling was rapid, and altho the supernatant liquid was quite opalescent, it could be poured rapidly through filter paper. When this filtrate was treated with more 1N HCl, .4 cc. caused a second precipitate to separate sharply. The quantity of the second fraction was considerably less than that of the first. When the clear supernatant liquid from the *second* fraction was again treated with 1N HCl, it became milky in appearance, but no further sharp precipitation could be obtained with HCl. Nevertheless it gave a considerable precipitate with phosphotungstic acid, indicating that the solution still contained either protein or complex peptides. Precisely the same results were obtained when a portion of the solution of slightly racemized protein *alone* was fractionated in the same manner. The first fraction came out at a pH of about 5.2 (as indicated by methyl red), while the color of the indicator in the case of the second precipitation indicated a pH of about 4.4.

Discussion

From the above experiments it appears that racemization of glutenin gives rise to more than one product. Even slight racemization makes it possible to precipitate two fractions from the alkaline solution, by bringing about the two respective necessary hydrogen-ion concentrations through successive additions of HCl. In view of the fact that the application of Halton's procedure to a number of glutenins and flour extracts invariably gave negative results, excepting in a case where slightly racemized protein was deliberately used, it is believed that Halton's "fractions" were secured from a glutenin which had become slightly racemized before he fractionated it. It is maintained by the writer that any given wheat flour contains but *one* glutenin, and that glutenin is a distinct chemical individual which can not be resolved into fractions.

Dakin (1912) attributes racemization of proteins by strong alkali to a keto-enol transformation within the protein molecule, as, $R\text{-}\acute{C}H\text{-}CO - \rightleftharpoons R\text{-}\acute{C} = C(OH) -$, whereby the central carbon atom loses its assymetry. The experiments here reported indicate that even with relatively slight racemization by dilute alkali, something more than an internal rearrangement of atoms occurs. In these experiments slight racemization of glutenin resulted in at least three "fractions." Two were precipitated at different hydrogen-ion concentrations, while

phosphotungstic acid precipitated more material which may be a peptide or a mixture of them. It would seem that racemization of proteins is accompanied by partial hydrolysis. Underhill and Hendrix (1915) have also obtained evidence that hydrolysis, as well as enolization of the protein molecule probably occurs when proteins are racemized by alkali. Apparently "racemized" proteins must be considered as protein derivatives.

Summary and Conclusions

1. Natural wheat flour glutenin is a chemical individual which can not be "fractionated" as is claimed by Halton (1924) without previous racemization.

2. Halton's conclusion that each flour contains two glutenin "fractions" of different chemical configuration, and that the flour strength will depend upon which of these fractions predominates, is considered to be founded upon insufficient and mistaken evidence.

3. Even slight racemization of glutenin gives rise to a second "fraction" which has a different isoelectric point from that of the natural glutenin, and it also results in other products which can be precipitated only by the use of heavy reagents such as phosphotungstic acid. "Racemization" even by dilute alkali appears to result in more than a mere internal rearrangement or enolization, and some hydrolysis probably always occurs. It appears probable that "racemized" proteins must be regarded as protein derivatives.

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A STUDY OF METHODS FOR MAKING PROTEIN TESTS ON WHEAT

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Beginning about ten years ago with individual mills and wheat buyers, the practice of buying wheat on protein content has advanced rapidly during the last three or four years, until practically 100 per cent of the hard wheat merchandised at Kansas City and other southwestern points has a protein test made on it from one to five times. This practice of buying on protein test has been taken up by other terminal markets, altho not to such a great extent. About 80 per cent of the hard wheats are now being tested for protein at St. Louis, Mo., about 40 per cent at Omaha, Neb.; and almost half of the wheat merchandised at Minneapolis, Minn., carries a protein or gluten label in some form. Many local mills at Buffalo, N. Y., and points in Pennsylvania buy wheat on known protein test.

Unfortunately, the manner of making protein tests and the results obtained by different laboratories have not kept step with the practice of trading on this factor. Many complaints are heard relative to inaccuracy in making these tests. Discrepancies of as much as 2 or 3 per cent have been noted when the same sample was considered.

In order to get some information on this outstanding subject, the grain division of the Bureau of Agricultural Economics made a survey of the practice of making protein tests at the most important markets where protein was a factor in merchandising wheat. The senior author in co-operation with J. H. Shollenberger, who conducts the milling investigations of the bureau, visited many of the mill, commercial and inspection department laboratories in the northwest and southwest wheat producing areas of the United States, to study methods and practices now being used in making protein tests. Samples of wheat representing the 1924 hard red spring and hard red winter crop were obtained at Kansas City and Minneapolis. These samples were submitted to 45 different laboratories with the request that they make protein tests and report results. Before submitting these samples, the wheat was thoroly cleaned, mixed, divided into approximately 50-gram portions, and placed in air-tight containers which were sealed with adhesive tape to insure uniformity of moisture content.

TABLE I

CRUDE PROTEIN RESULTS OBTAINED BY VARIOUS LABORATORIES ON TWELVE SAMPLES OF WHEAT

Laboratory number	Identification numbers of samples submitted: 1 to 40	41 to 80	81 to 120	121 to 160	161 to 200	201 to 240	241 to 260	261 to 280	281 to 300	301 to 330	331 to 356	357 to 385
Research or Experimental Laboratories												
1	10.53	11.74	12.71	12.59	12.60	15.39	12.25	12.65	14.31	10.15	10.37	10.94
2	10.54	11.63	12.82	12.48	12.60	15.45	12.20	12.71	14.36	10.20	10.32	10.94
3	10.60	11.74	12.83	12.60	12.65	15.50	12.08	12.65	14.31	10.20	10.37	10.83
4	.....	.....	.....	.....	.....	.....	12.14	12.71	14.34	10.85	10.59	10.93
5	10.65	11.60	12.82	12.56	12.58	15.40	.....	.....	.....	.....	.....	.....
6	10.60	11.64	12.84	12.48	12.24	15.32	.....	.....	.....	.....	.....	.....
7	.....	.....	.....	.....	.....	.....	11.43	12.20	13.46	10.00	10.26	10.83
42	10.60	12.00	13.02	12.83	12.88	15.56	12.31	13.05	14.25	10.55	10.80	11.09
Average	10.59	11.72	12.84	12.59	12.59	15.44	12.07	12.68	14.17	10.32	10.45	10.93
Maximum	10.65	12.00	13.02	12.83	12.88	15.56	12.31	13.05	14.36	10.85	10.80	11.09
Minimum	10.53	11.60	12.71	12.48	12.24	15.32	11.43	12.20	13.46	10.00	10.26	10.83
Range	0.12	0.40	0.31	0.35	0.64	0.24	0.88	0.85	0.90	0.85	0.54	0.26
Inspection Department Laboratories												
8	10.40	11.36	12.40	12.42	12.44	15.28	.....	.....	.....	.....	.....	.....
9	10.64	11.68	12.66	12.54	12.62	15.40	.....	.....	.....	.....	.....	.....
10	10.68	11.78	12.62	12.60	12.64	15.48	.....	.....	.....	.....	.....	.....
11	10.72	11.76	12.68	12.52	12.52	15.08	.....	.....	.....	.....	.....	.....
12	10.36	11.49	12.89	12.62	12.42	15.44	12.07	12.55	14.13	10.29	10.38	10.65
Average	10.56	11.61	12.65	12.54	12.53	15.34	12.07	12.55	14.13	10.29	10.38	10.65
Maximum	10.72	11.78	12.89	12.62	12.64	15.48	.....	.....	.....	.....	.....	.....
Minimum	10.36	11.36	12.40	12.42	12.42	15.08	.....	.....	.....	.....	.....	.....
Range	0.36	0.42	0.49	0.20	0.22	0.40	.....	.....	.....	.....	.....	.....

TABLE I—*Continued*

CRUDE PROTEIN RESULTS OBTAINED BY VARIOUS LABORATORIES ON TWELVE SAMPLES OF WHEAT

Laboratory number	Identification numbers of samples submitted: 1 to 40	41 to 80	81 to 120	121 to 160	161 to 200	201 to 240	241 to 260	261 to 280	281 to 300	301 to 330	331 to 356	357 to 385
Mill and Elevator Laboratories												
13	10.58	11.70	12.56	12.76	12.75	15.56	.....	.....	.....	.....	.....	.....
14	10.54	11.58	12.78	12.46	12.62	15.09	.....	.....	.....	.....	.....	.....
15	10.61	11.50	12.73	12.43	12.64	15.43	.....	.....	.....	.....	.....	.....
16	10.70	11.75	12.70	12.65	12.85	15.45	12.25	12.95	14.35	10.40	10.55	11.00
17	10.34	11.58	12.48	12.44	12.56	15.16	.....	.....	.....	.....	.....	.....
18	10.42	11.59	12.69	12.57	12.53	15.34	.....	.....	.....	.....	.....	.....
19	10.71	11.90	12.80	12.54	12.92	15.70	.....	.....	.....	.....	.....	.....
20	10.50	11.50	12.52	12.44	12.50	15.32	.....	.....	.....	.....	.....	.....
21	10.56	11.76	12.72	12.64	12.64	15.40	12.16	12.72	14.16	10.24	10.32	10.96
22	10.53	11.97	12.53	12.65	12.21	15.20	.....	.....	.....	.....	.....	.....
23	10.60	11.75	12.70	12.45	12.65	15.45	.....	.....	.....	.....	.....	.....
24	10.42	12.02	13.08	12.58	12.38	15.80	.....	.....	.....	.....	.....	.....
25	10.51	11.64	12.70	12.48	12.46	15.36	.....	.....	.....	.....	.....	.....
26	10.80	11.88	13.16	12.56	12.64	15.52	.....	.....	.....	.....	.....	.....
27	.....	.....	.....	.....	.....	.....	12.49	12.49	14.12	10.37	10.37	11.31
28	.....	.....	.....	.....	.....	.....	12.42	13.26	14.20	10.60	10.48	11.16
29	10.79	11.93	13.00	12.84	12.88	15.73	12.39	13.00	14.57	10.56	10.64	11.17
30	10.93	11.95	12.90	12.63	12.60	15.68	12.40	12.90	14.30	10.55	10.50	11.18
31	.....	.....	.....	.....	.....	.....	12.36	13.02	14.62	10.50	10.76	11.26
43	.....	.....	.....	.....	.....	.....	12.58	12.73	14.40	10.57	10.85	10.93
Average	10.60	11.76	12.75	12.57	12.61	15.45	12.38	12.88	14.34	10.47	10.56	11.12
Maximum	10.93	12.02	13.16	12.84	12.92	15.80	12.58	13.26	14.62	10.60	10.85	11.31
Minimum	10.34	11.50	12.48	12.43	12.21	15.09	12.16	12.49	14.12	10.24	10.32	10.93
Range	0.59	0.52	0.68	0.41	0.71	0.71	0.42	0.77	0.50	0.36	0.53	0.38

TABLE I—*Continued*

CRUDE PROTEIN RESULTS OBTAINED BY VARIOUS LABORATORIES ON TWELVE SAMPLES OF WHEAT

Laboratory number	Identification numbers of samples submitted: 1 to 40	41 to 80	81 to 120	121 to 160	161 to 200	201 to 240	241 to 260	261 to 280	281 to 300	301 to 330	331 to 356	357 to 385
Commercial Laboratories												
32	10.44	11.88	12.76	12.68	12.68	15.32	.....	.....	.....	.....	.....	.....
33	10.81	11.90	12.69	12.61	12.81	15.68	.....	.....	.....	.....	.....	.....
34	10.88	11.70	12.72	12.64	12.64	15.28	12.40	12.78	14.10	10.48	10.44	11.26
35	10.53	11.65	12.77	12.57	12.77	15.48	.....	.....	.....	.....	.....	.....
36	10.80	11.80	12.80	12.50	12.70	15.60	12.60	12.60	14.30	10.40	10.40	11.10
37	11.00	12.14	13.11	13.00	13.11	16.04	12.32	12.94	14.62	10.37	10.72	11.00
38	.....	.....	.....	.....	.....	.....	12.47	13.15	14.51	10.49	10.77	11.70
39	10.85	11.90	12.92	12.75	12.88	16.00	12.52	12.80	14.68	10.42	10.62	11.05
40	10.80	11.75	12.75	12.75	12.90	15.55	12.25	13.05	14.45	10.40	10.50	11.25
41	10.45	11.77	12.43	12.46	13.05	12.89	11.92	12.04	14.13	10.20	11.01	10.98
Average	10.73	11.83	12.77	12.64	12.84	15.32	12.33	12.77	14.40	10.39	10.64	11.19
Maximum	11.00	12.14	13.11	13.00	13.11	16.04	12.60	13.15	14.68	10.49	11.01	11.70
Minimum	10.44	11.65	12.43	12.46	12.64	12.89	11.92	12.04	14.10	10.20	10.40	10.98
Range	0.56	0.49	0.68	0.54	0.47	3.15	0.68	1.11	0.58	0.29	0.61	0.72
Summary for all Groups												
Average	10.62	11.75	12.76	12.59	12.65	15.40	12.26	12.78	14.30	10.40	10.55	11.07
Maximum	11.00	12.14	13.16	13.00	13.11	16.04	12.60	13.26	14.68	10.85	11.01	11.70
Minimum	10.34	11.36	12.40	12.42	12.21	12.89	11.43	12.04	13.46	10.00	10.26	10.65
Range	0.66	0.78	0.76	0.58	0.90	3.15	1.17	1.22	1.22	0.85	0.75	1.05
Averages of the Four Groups												
Research or experimental	10.59	11.72	12.84	12.59	12.59	15.44	12.07	12.68	14.17	10.32	10.45	10.93
Inspection department	10.56	11.61	12.65	12.54	12.53	15.34	.....	.....	.....	.....	.....	.....
Mill and elevator	10.60	11.76	12.75	12.57	12.61	15.45	12.38	12.88	14.34	10.47	10.56	11.12
Commercial	10.73	11.83	12.77	12.64	12.84	15.32	12.40	12.77	14.40	10.39	10.64	11.19

*Acknowledgment is made to J. H. Shollenberger who helped submit these samples and who also compiled the data in this table.

The data obtained from this cooperative study are given in Table I. As shown in the table, 40 different portions were taken from the same bulk sample, so that Nos. 1 through 40, 41 through 80, 81 through 120, etc., refer to different bulk samples. Samples numbered 1 to 240 comprise wheats of the hard red winter crop, and samples 241 to 385, inclusive, represent wheats of the hard red spring crop. The collaborating laboratories were grouped into four divisions: Research or Experimental laboratories, Inspection Department laboratories, Mill and Elevator laboratories, and Commercial laboratories. A study of this table throws some interesting light upon the accuracy of protein tests.

Considering first the summary section for all groups of laboratories, we find that the maximum difference found with any one sample was 3.15% and the minimum difference found was 0.58%. These figures are much larger than one usually associates with good technic in making protein tests and are considerably larger than that allowed by either the Kansas City or Omaha boards of trade, viz., 0.12 and 0.20 per cent. There was a greater range with wheats within the hard red spring class than with those of the hard red winter class, if we eliminate the difference of 3.15% in samples 201-240, which throws the average for this sample of wheat radically out of line. This large difference was obtained by only one investigator and it is believed that there must have been a mistake in his calculations as his other analyses were within the range obtained by the other laboratories. Considering each division by itself, it was found that there were lesser differences in the data submitted by the inspection department and by the research laboratories than in any other group of co-operators. The greatest differences were obtained by the commercial laboratories, with the mill and elevator laboratories next.

It is difficult to explain why such large differences should be obtained without knowing something of the methods used by each of the collaborators. A questionnaire was therefore sent, asking for information relative to the methods used and for such data as size of sample ground, digestion mixture, time of digestion, character of still, nature and amount of receiving acids, etc.

The data on this subject are given in Table II. The size of the samples ground to make the protein test varied from 6 to 60 grams. One collaborator did not grind his sample, but made the test on the whole seed. Roughly speaking, 65 per cent of the laboratories digested a one-gram sample and 35 per cent digested a two-gram sample. The amount of acid used in the digestion mixture varied from 15 to 25 cc.

with the laboratories using a one-gram sample. Thirty per cent of those digesting a one-gram sample used 25 cc.; 25 per cent, 15 to 18 cc.; and 38 per cent, 20 cc. Of the laboratories using a two-gram sample for digestion, 40 per cent used 25 cc. of acid; 40 per cent, 30 cc.; and 18 per cent, 20 cc.

The catalytic agents used were numerous—sodium sulphate, potassium sulphate, metallic mercury, yellow oxide of mercury, red oxide of mercury, mercuric sulphate, metallic copper, and copper sulphate. Approximately 63 per cent of the laboratories used sodium sulphate in amounts varying from 3 to 15 grams, 35 per cent using potassium sulphate in the same amounts. Considering the mercuric catalysts, 18 per cent used metallic mercury; 10 per cent, yellow oxide of mercury; 8 per cent, red oxide of mercury; and 18 per cent, mercuric sulphate. The lowest amount of mercury used was 0.5 gram and the highest, 1 gram. The usual amount of any form of mercury used was 0.5 to 0.7 gram. Copper sulphate in amounts varying from 0.05 gram to 2 grams was used by 47 per cent of the laboratories. The great majority, however, used approximately 0.2 gram. Three collaborators used metallic copper in the same ratio that copper sulphate was being used.

The Kjeldahl (8) method or modifications thereof was used by 24 per cent of the laboratories. Forty per cent used the Gunning (9) method; 18 per cent, the method developed by the Kansas City Protein Referee Board (4); and the remainder used methods peculiar to themselves. The time of digesting the samples varied from 25 to 180 minutes. Both electricity and gas were used. The average time consumed with the gas heaters was 75 minutes; with the electric heaters, 50 minutes. Altho the differences are not very great, a greater range and a higher average protein content were found by the laboratories using electricity than by those using gas.

The receiving acids were three—sulphuric, hydrochloric, and boric. Expressed as acid sufficient to hold one milligram of nitrogen, a sufficient amount was used to hold from 14 to 122.5 milligrams. The strength of the hydrochloric and sulphuric acids varied from N/14 to N/2, whereas the usual 4% solution of boric acid was used. When it is known that wheat will vary in protein content from 7 to approximately 20%, it is evident that acid equivalent to 35 milligrams of nitrogen must be present to hold the nitrogen released from all types of samples, when a one-gram sample is digested. These data show that few of the laboratories that submitted figures on this subject are using insufficient acid to hold these amounts of nitrogen.

TABLE II

DATA PERTAINING TO THE MAKING OF PROTEIN TESTS AS GIVEN BY 38 COLLABORATORS

Collaborator number	Grams of sample ground	Grams of sample digested	Acid used for digestion	Catalytic agents: Mercury†	Copper*	K_2SO_4	Na_2SO_4	Heat	Time of digestion	Strength and nature of receiving acids	Nitrogen receiving acids will hold	Mercury precipitant	Acid or alkali standardized as
			cc.	Grams	Grams	Grams	Grams		Minutes		Mgms.		
					Catalytic agents								
1	50	2	30	.70Y	....	10–12	.....	Gas	50	.20 N HCl	70	$Na_2S_2O_3$	AgCl
2	50	2	30	.70Y	....	10–12	.....	Gas	50	.20 N H_2SO_4	70	$Na_2S_2O_3$	$BaSO_4$
3	50	2	30	.70Y	....	10–12	.....	Gas	50	.20 N HCl	70	$Na_2S_2O_3$	$KHC_8H_4O_4$
4	50	2	25	....	.20S	.....	8	Gas	150	HCl	..	....	AgCl
									A. C.‡				
5	40	2	20	....	.28S	.....	10	Gas	60	.20 N H_2SO_4	56	....	Cross titer
6	U.G.§	U.G.§	30	.50M	....	.....	15	Gas	180	.25 N H_2SO_4	84	K_2S	$BaSO_4$
7	100	1	25	....	2.00S	.....	7	Gas	90	.07 N H_2SO_4	30	....	$BaSO_4$
8	25	1	25	.80S	.20S	10	.....	Elec.	40	.....	..	K_2S	Oxalic, Benzoic, Constant Boiling Point HCl
9	50	1	15	.70Y	....	.....	10	Elec.	30‖	.10 N H_2SO_4	35	K_2S	Benzoic
10	75	1	18	....	.26S	.....	8–9	Elec.	40–60	.....	..	....	Benzoic, $BaSO_4$, Potassium Bitartrate
11	50	1	20	....	.10M	.....	10	Elec.	35	H_2SO_4	..	....	Oxalic, Benzoic
12	50	1	25	.50S	....	.....	6.40	Elec.	50	.125N H_2SO_4	17.5	K_2S	$BaSO_4$
13	50	1	20	.55S	.11S	.....	.....	Elec.	50	.20 N H_2SO_4	35	K_2S	Oxalic
14	15–20	1	15	....	.05S	.....	3–31.50	Elec.	80	H_2SO_4	..	....	Benzoic
15	50	1	20	.50S	.10S	.....	6.40	Elec.	50	Boric	70¶	K_2S	$BaSO_4$ Constant boiling point HCl
16	50	1	20	.75S	.10S	.....	6.25	Gas	60	Boric	70	Na_2S	Oxalic
17	50	1	20	....	....	.....	7	Elec.	45	.20 N H_2SO_4	35	....	Benzoic
18	20	1	20	.50M	....	.....	5	Elec.	60	H_2SO_4	..	K_2S	$BaSO_4$
19	25	1	25	.55S	.11S	.....	6.40	Elec.	35	Boric	70	K_2S	$BaSO_4$, Cuccinic
20	20	1	25	....	.20S	10	.....	Elec.	40	.10 N H_2SO_4	35	....	Oxalic, Benzoic
21	20–25	1	15	.70–.90M	....	7–8	.....	Gas	50–60	H_2SO_4	..	K_2S	Na_2CO_3
22	25	1	20	.50Y	....	.....	5.00	Gas	60	H_2SO_4	..	K_2S	$BaSO_4$

TABLE II—*Continued*

DATA PERTAINING TO THE MAKING OF PROTEIN TESTS AS GIVEN BY 38 COLLABORATORS

Collaborator number	Grams of sample ground	Grams of sample digested	Acid used for digestion	Mercury†	Copper*	K_2SO_4	Na_2SO_4	Heat	Time of digestion	Strength and nature of receiving acids	Nitrogen receiving acids will hold	Mercury precipitant	Acid or alkali standardized as
			cc.	Grams	Grams	Grams	Grams		Minutes		Mgms.		
23	50	1	20	.50S	.10S	.....	6.40	Elec.	25	Boric	70	K_2S	Oxalic, Succinic
24	..	1	25	....	.05M	.....	9.00	Gas	75	.10 N H_2SO_4	35	....	Benzoic
25	25	1	18	....	....	2–3	.....	Gas	120	.10 N H_2SO_4	14	K_2S	Oxalic
26	..	1	15	....	.08S	.....	8–10	Gas	50	.....	..	....	.....
27	6	2	25	.70R	....	10	.....	Gas	90	.10 N H_2SO_4	70	K_2S	Na_2SO_4
29	60	1	20	.60R	....	.....	6	Elec.	45	H_2SO_4	..	$Na_2S_2O_3$	$BaSO_4$
30	10	2	25	....	.20S	.....	8	Gas	90	H_2SO_4	..	....	$BaSO_4$
31	..	2	25	....	.10M	.....	10	Gas	90	.....	..	....	.....
33	60	1	17.5	....	.20S	8	.....	Gas	45–60	.....	..	....	Oxalic
34	50	2	20	.50	....	8	.....	Gas	45	.50 N H_2SO_4	122.5	Na_2S	$BaSo_4$
35	100	2.5	25	1.00	....	.....	3.50	Gas	U.C.♦	.50 N HCl	105	Na_2S	AgCl
36	15	1	15	.70	....	.....	4.00	Elec.	30	H_2SO_4	..	Na_2S	$BaSO_4$, K-Acid Tartrate
37	50	1	25	....	.20S	8	.....	Gas	45	.20 N H_2SO_4	56	....	$BaSO_4$, Na_2CO_3
38	125	2	30	....	1.00S	.....	10	Gas	60♦	.10 N H_2SO_4	56	....	$BaSO_4$
39	150–200	2	25	.60	....	.....	.....	Gas	30–35	.25 N H_2SO_4	52.5	K_2S	$BaSO_4$, Na_2CO_3
45	15–20	2	30	....	.20S	.....	3–4	Elec.	90	.10 N H_2SO_4	56	....	$BaSO_4$

*M=Metallic copper; S=Copper sulphate.

†M=Metallic mercury; Y=Yellow oxide of mercury; R=Red oxide of mercury; S=Mercuric sulphate.

‡After clearing.

§Unground.

‖Burners hot to start.

¶According to scales and Harrison.

♦Until colorless.

As many variables entered into the final analysis, it was almost impossible, in order to eliminate and study each factor separately, to determine which was responsible for the differences in the collaborative work. Investigations were therefore planned to discover the causes of some of the differences. These investigations were carried forward in two sections, the first having to do with the methods of digesting the samples, and the second relating to the technic of distilling. The outstanding points for study as brought out by the data in Table II are: Methods, time of digestion, quantity and nature of the catalytic agents, correct standardization of the receiving or titrating solutions, preparation of the sample for analysis, nature and volume of the receiving acids, and care in distillation.

Digestion Studies

In planning this work, recognition was given to the importance of time of digestion. It is evident that this will vary, depending upon the intensity of the heat. For these studies, therefore, three different intensities of heat were chosen. These are described as low, medium, and high heat. The standards for these heats were established as follows: 200 cc. of distilled water was placed in 500-cc. Kjeldahl flasks similar to those used in the digestion process, and brought to a boil. The heaters and burners were so adjusted that with the low heat 50 cc. of water evaporated in 20 minutes; with the medium heat, 100 cc.; and with the high heat, 150 cc. In order to keep the heat intensities at the same degree so that they would not vary from time to time and from day to day, each gas burner was set to give the same amount of heat, and it was kept this way by means of a gas pressure regulator. Frequent check tests were made to keep the heat constant.

Acid Necessary for Digestion of Wheat Samples

It will be seen by consulting Table II that the acid used by the various collaborators varied considerably, regardless of size of sample. Experiments were made, therefore, to determine how much acid was needed completely to digest wheat samples.

Into 500-cc. volumetric flasks were measured 20-cc. portions of sulphuric acid, specific gravity, 1.82, and made to volume. The acid present was determined by titrating against a known solution of potassium hydroxid. Twenty-cc. portions of the same acid were then placed in 500-cc. Kjeldahl flasks with 10 grams of K_2SO_4 and 0.7 gram yellow mercuric oxide, and the mixture was heated for the time necessary to digest a sample of wheat. This time varied with the heat intensity. With low heat, two hours; with medium heat, an hour

and 15 minutes; and with high heat, 45 minutes. At the end of each heating period, the acid was transferred to a 500-cc. volumetric flask, made to volume, and the acid content determined as before. Ten determinations were made on each heating time to overcome any minor differences in the heat of the burners. The difference in amount of acid present before and after heating was considered as the amount volatilized by heating. Tests of acid losses were made, using the Kjeldahl (8) mixture with sodium sulphate instead of potassium sulphate. Tests were also made using the Gunning (9) mixture, and the mixture recommended by the Kansas City Protein Referree Board (4).

To determine the acid loss due to oxidation of the wheat samples, one-gram portions of wheat from 10 different samples, varying in protein content from 8.09 to 18.70%, were digested at medium heat for 75 minutes (complete oxidation) with the Kjeldahl mix (10 K_2SO_4 + .7 HgO). At the end of this period, the acid remaining was transferred to 500-cc. volumetric flasks and the acid content determined. Subtracting this value from the acid remaining after heating gave the acid loss due to digestion of the one-gram sample.

The experiment was repeated with three samples using 25 cc. of acid and 2 grams of sample, measuring the acid content before and after heating, and after heating with the samples.

The data from these investigations are given in Table III.

These data bring out the following points: Approximately 2 to 3.5 cc. of acid are lost by heating the acid with the catalytic reagents. The smallest loss was obtained at the lowest intensity of heat and the loss increased as the heat was increased. No greater heat appears to be developed by any one of the three formulae as measured by subsequent acid loss.

The acid loss due to oxidation of the samples, when a one-gram sample was digested, varied from 3.1 to 4.5 cc. The average loss was 3.8 cc. A two-gram sample requires twice as much acid as a one-gram sample. The total acid loss due to heating of a medium nature and oxidation of a one-gram sample was 6 cc. Not quite as much, 5.8 cc., was lost when the heat intensity was low, whereas 7.3 cc. was lost when a high heat was used.

Knowing the acid losses as given, one can then calculate the optimum amount of acid to use if further information is at hand regarding the acid necessary to hold the ammonia nitrogen split off from the protein molecule. To determine this, a portion of the work of Paul and Berry (10) was repeated and the data are given in Table IV.

TABLE III

SULFURIC ACID* LOSSES DUE TO HEATING AND TO OXIDATION OF WHEAT SAMPLES

Treatment number	Treatment	Protein	Intensity of heat	Acid used	Acid lost by heating	Acid lost by oxidation	Total loss due due to heat and oxidation
		Per cent		cc.	cc.	cc.	cc.
1	Acid — only — starting	.....	No heat	20	...	...	...
2	10 gr. K_2SO_4 + .7 gr. HgO	.....	Low	20	2.0	...	...
3	10 gr. K_2SO_4 + .7 gr. HgO	.....	High	20	3.5	...	...
4	10 gr. K_2SO_4 + .7 gr. HgO	.....	Medium	20	2.4	...	...
5	10 gr. Na_2SO_4 + .7 gr. HgO	.....	Medium	20	2.3	...	...
6	10 gr. Na_2SO_4 + .2 gr. $CuSO_4$	.....	Medium	20	2.5	...	...
7	7 gr. Na_2SO_4 + .1 gr. $CuSO_4$ + 0.5 gr. $HgSO_4$	.....	Medium	20	2.5	...	...
8	Treatment 4 — 1 gr. 11,998	10.66	Medium	20	2.4	3.8	6.2
9	" 4 — 1 gr. 12,002	12.88	Medium	20	2.4	3.4	5.8
10	" 4 — 1 gr. 12,003	15.45	Medium	20	2.4	3.4	5.8
11	" 4 — 1 gr. 12,005	12.77	Medium	20	2.4	3.4	5.8
12	" 4 — 1 gr. 12,006	14.53	Medium	20	2.4	3.1	5.5
13	" 4 — 1 gr. 12,007	10.20	Medium	20	2.4	4.5	7.9
14	" 4 — 1 gr. 12,376	8.26	Medium	20	2.4	3.6	6.0
15	" 4 — 1 gr. 12 868	8.09	Medium	20	2.4	3.7	6.1
16	" 4 — 1 gr. 12,878	17.73	Medium	20	2.4	3.5	5.9
17	" 4 — 1 gr. 12,883	18.07	Medium	20	2.4	3.4	5.8
18	" 4 — 2 gr. 12,002	12.88	Medium	25	3.0	6.7	6.4
19	" 4 — 2 gr. 12,606	14.53	Medium	25	3.0	7.0	6.5
20	" 4 — 2 gr. 12,376	8.26	Medium	25	3.0	6.6	6.3

Average acid lost from heat and oxidation, with 20 cc. of acid.....6.1
Average acid lost from heat and oxidation, with 25 cc. of acid.....6.4

*Specific gravity of acid, 1.82 at 25° C.

TABLE IV

SULPHURIC ACID NECESSARY TO HOLD THE AMMONIA PRESENT DURING THE DIGESTION PERIOD

Concentrated sulphuric acid in flask	Protein present	Difference
Series I		
	Per cent	Per cent
$(NH_4)_2SO_4$ as protein added.....	13.57	.....
30 cc. H_2SO_4 + 10 gr. K_2SO_4 + .7 gr. Yellow HgO.....	13.62	.05
25 cc. " + " " + " " " "	13.57	.....
20 cc. " + " " + " " " "	13.57	.....
15 cc. " + " " + " " " "	13.57	.....
10 cc. " + " " + " " " "	13.62	.05
5 cc. " + " " + " " " "	3.42	10.22
Series II		
$(NH_4)_2SO_4$ as protein added.....	13.57	.....
30 cc. H_2SO_4 + 10 gr. Na_2SO_4 + .7 gr. Yellow HgO.....	13.57	.....
25 cc. " + " " + " " " "	13.57	.....
20 cc. " + " " + " " " "	13.57	.....
15 cc. " + " " + " " " "	13.57	.....
10 cc. " + " " + " " " "	13.62	.05
5 cc. " + " " + " " " "	3.76	9.71

This experiment was made with both sodium and potassium sulphate as catalytic agents, as it was felt by some laboratory personnel that sodium sulphate induced greater acid losses than potassium sulphate. This experiment was carried on at the greatest intensity of heat obtainable for one hour.

The data in Table IV substantiate fully the claims of Paul and Berry, namely, that it is necessary to have at least 10 cubic centimeters of acid present to hold all the nitrogen.

From the data as given in Tables III and IV, it is evident that at least 10 cc. of acid is necessary to hold the nitrogen formed during the oxidation process at all intensities of heat. It is necessary to have 6 cc. of acid to care for heat and oxidation losses when one-gram samples are digested at a medium intensity of heat, 5.8 cc. at low heat, and 7.3 cc. at high heat, making the total quantity of acid necessary at the three intensities of heat: 15.8 cc. at low heat, 16 cc. at medium heat and 17.3 cc. at high heat. When a two-gram sample is used, 4 cc. additional acid must be used.

These acid losses will be subject to slight variations, depending upon the draft in the fume tubes or hood. Strong drafts will add about one cc. to the total given. Strange to say, a strong draft likewise reduces the oxidation time approximately 10 per cent. It is therefore recommended that 20 cc. of sulphuric acid be used with a one-gram sample of wheat and 25 cc. with a two-gram sample.

Further observations were made noting the effect of 15, 20, 25, and 30 cc. of concentrated acid upon the rapidity of oxidation of one-gram samples. Using the greatest intensity of heat, 15 cc. of acid causes too much frothing. With 20 cc of acid the digest cleared in 6½ minutes; with 25 cc., in 8½ minutes; and with 30 cc., in 13 minutes. At a high intensity of heat, 30 cc. of acid is too much, as there is considerable bumping and spurting during the digestion process, which, no doubt, would result in material losses. Protein determinations made at the end of 15 minutes, or before oxidation was complete, showed that oxidation had progressed further with 20 cc. than with any of the other quantities of acid. It is reasonable to suppose that if this takes place at high heat it will also take place at the lower intensities of heat.

Efficiency of Heat and Catalytic Agents on Oxidation of Wheat Samples with Sulphuric Acid

As was pointed out, the periods of digestion and the catalytic agents were numerous within the formulae used by the various collaborators. No standard time or quantity of reagent was employed by the majority. The question arose as to the efficiency of heat and

of these reagents in varying amounts, as an aid to the rapid digestion of wheat samples.

The influence of heat on the oxidation process was studied by digesting wheats of high and low protein content for 30, 60, and 90 minutes with 20 cc. of concentrated sulphuric acid, at high, low, and medium intensities of heat. The data from this study are given in Table V.

TABLE V

INFLUENCE OF HEAT AND ACID ON PROTEIN TEST RESULTS

Intensity of heat	Sample number	Time, minutes: 30 — Protein found	30 — Digested	60 — Protein found	60 — Digested	90 — Protein found	90 — Digested
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
High	6	14.08	91.55	14.42	93.76	14.53	94.47
"	8	10.49	92.02	10.66	93.51	10.72	94.04
Medium	6	14.02	91.15	14.31	93.04	14.36	93.37
"	8	10.26	90.00	10.49	92.02	10.60	92.98
Low	6	13.91	90.44	14.19	92.33	14.26	92.78
"	8	10.20	89.47	10.36	90.87	10.54	92.45

These data show that heat is **the most important** factor to be considered in digesting wheat samples. As great an oxidation can be obtained in 30 minutes with a high degree of heat as can be obtained in 90 minutes at low heat.

At all intensities of heat with the two samples tested, over 90% of the protein nitrogen was in the form of ammonia within 30 minutes. In 90 minutes, 94.25% of the protein nitrogen was in this form at high heat, 93.2% at medium heat, and 92.5% at low heat.

These data also indicate that samples of low protein content digest at a slower rate than those of high protein content.

By increasing the heat 50% above the maximum heat used in the above discussion, it was found that over 99% of the protein nitrogen in samples of wheat could be transformed into ammonia in less than 15 minutes. The results, however, were not regular, varying within 1% of complete oxidation with different samples. Moreover, at this intensity of heat, the flasks were so hot that they were very difficult to handle.

In studying the effect of catalytic agents on the oxidation process, a shorter period of time was chosen than was necessary to complete the oxidation of the wheat samples, so that the heat factor would not mask the effects of the reagent studied. Two samples of wheat were used, one of high, the other of low protein content, and the results from each sample were averaged. The studies were made at low and medium intensities of heat. A one-gram sample and 20 cc.

of concentrated acid were used throughout, the catalyst being changed to suit the occasion. These data are shown in Table VI. In this table a value of 100 has been given to the results when the sample was digested with acid alone and when catalysts were used the results are expressed in terms of this value.

These data show that there is no advantage in choosing between red and yellow oxide of mercury in any amount. The best amount to use seems to be 0.5 gram. No advantage is gained by doubling the amount of these reagents. Mercuric sulphate as a catalyst gave slightly lower results.

TABLE VI

VALUE OF CATALYTIC AGENTS ON PROTEIN TEST RESULTS

Mercury salts				K_2SO_4 and Na_2SO_4			$CuSO_4$*	
Grams used	Yellow HgO	Red HgO	$HgSO_3$	Grams used	K_2SO_4	Na_2SO_4	Grams used	$CuSO_4$
	Per cent	Per cent	Per cent		Per cent	Per cent		Per cent
.0	100	100	100	0	100	100	.0	100
.1	102	103	102	3	103	103	.1	100
.3	104	104	103	5	104	103	.3	101
.5	105	105	104	7	104	104	.5	102
.7	105	105	104	10	105	104	1.0	101
1.0	105	105	104	15	105	105	2.0	100
...	...	...	...	..	...	...	3.0	100

*Low intensity of heat only.

At low heat, copper sulphate has no marked value as a catalyst. In fact, large amounts of this reagent reduced the rapidity of oxidation. At higher intensities of heat, however, this catalyst seems to work well.

Ten grams seems to be the best weight to use of either sodium or potassium sulphate, neither having any chemical advantage over the other. In choosing between these two salts as catalytic agents, the tendency of sodium sulphate to form an acid cake with sulphuric acid must be considered, along with the cost of these two reagents. Unless sodium sulphate digests are dissolved in water soon, time will be lost in getting the salt into solution. To overcome this, it was found that a mixture of 40% sodium sulphate and 60% potassium sulphate will not form an acid cake.

Objection might be raised that a short period of time, such as 30 minutes, would not be sufficient for the catalysts to exert their full effect. To meet this objection, the heating time was extended to one hour with two of the catalysts.

From the data given in Table VII it is seen that the effect of the catalyst was such that it reached its level immediately and remained there during the period of the test. From this it can be concluded

that the full effect of catalytic agents is immediate and that further breaking up of the protein molecule is due to extended heating.

TABLE VII

INFLUENCE OF TIME ON ACTIVITIES OF CATALYTIC AGENTS

	Time of digestion			
	30 minutes		60 minutes	
Red HgO, grams	Nitrogen as cc. N/10 alkali	Difference, cc. N/10 alkali	Nitrogen as cc. N/10 alkali	Difference, cc. N/10 alkali
.0	17.25	...	17.89	...
.30	17.92	.67	18.59	.70
.70	18.02	.77	18.69	.80
1.00	17.97	.72	18.67	.78

	Time of digestion			
	30 minutes		60 minutes	
Yellow HgO, grams	Nitrogen as cc. N/10 alkali	Difference, cc. N/10 alkali	Nitrogen as cc. N/10 alkali	Difference, cc. N/10 alkali
.0	17.25	...	17.89	...
.30	17.92	.67	18.58	.69
.70	18.04	.79	18.58	.68
1.00	18.04	.79	18.67	.78

Influence of Size of Sample Digested on Protein Test Results

As one- and two-gram samples were used in this study, tests were made to note the influence of size of sample on results. Both one- and two-gram portions were digested for a time sufficient to afford complete oxidation. These data are given in Table VIII.

TABLE VIII

COMPARISON OF ONE-GRAM WITH TWO-GRAM SAMPLES ON PROTEIN TEST RESULTS

	Ranges in percentage of protein						
	10-10.99	11-11.99	12-12.99	13-13.99	14-14.99	15-15.99	16-20
				One-gram sample			
	10.26	11.34	12.71	13.00	14.65	15.39	19.55
	10.60	11.00	12.77	13.11	14.19	15.10	18.81
	10.15	11.80	12.77	13.39	14.02	14.90	18.29
	10.66	11.97	12.90	13.45	14.60	14.90	17.90
	10.32	11.34	12.03	13.79	14.31	.....	.....
	.....	11.97	12.60	13.51	14.82	.....	.....
	.....	.....	12.54	13.05	.....	.....	.....
	.....	.....	12.37	13.39	.....	.....	.....
Average	10.39	11.57	12.58	13.34	14.43	15.07	18.64
				Two-gram sample			
	10.22	11.34	12.75	13.08	14.71	15.45	19.55
	10.60	10.94	12.77	13.12	14.25	15.28	18.81
	10.15	11.75	12.78	13.39	14.08	15.03	17.95
	10.66	11.91	12.94	13.40	14.65	15.10	18.34
	10.32	11.34	12.00	13.85	14.31	.....	.....
	.....	11.97	12.60	13.62	14.83	.....	.....
	.....	.....	12.54	13.11	.....	.....	.....
	.....	.....	12.40	13.45	.....	.....	.....
Average	10.39	11.54	12.59	13.37	14.47	15.21	18.66
Difference of average	0.00	—.03	+.01	+.03	+.04	+.14	+.02

Samples were chosen having protein contents of from 8 to 18% in 1 per cent ranges.

These data show on the average that there is a slight advantage in using a two-gram sample, especially with wheats containing the higher percentages of protein. This advantage is not sufficiently great to warrant its use, as it takes approximately 20% longer completely to digest a two-gram sample. When this factor is considered in the light of a laboratory making several hundred tests a day, the total additional time necessary to oxidize two-gram samples will be considerable.

It is apparent also that the large differences obtained by the 38 collaborators can not be attributed to size of sample digested.

Influence of Method, Time, and Sample on Protein Test Results

It will be remembered that the time of digestion given to the samples by the various collaborators varied from 25 to 180 minutes. Less time was consumed by those using electricity as a source of heat than by those using gas. Three distinct methods were used: The Kjeldahl (8) method, the Gunning (9) method, and the Kansas City Protein Referee Board (4) method. Large variations were also obtained by many laboratories with some of the spring wheats. Experiments were planned, therefore, to study the relation of these variables upon the protein test results. In this study the three different intensities of heat, described previously, were used. Three different methods were used, with wheats having a high, a medium, and a low protein content. Samples from the hard red spring crop were chosen, as greater difficulty was experienced in obtaining uniform results from these samples. All samples were ground to pass through a No. 36 grits-gauze sieve. As shown in Table IX, the time was varied from 15 minutes to 2 hours in 15-minute intervals. Tests were run in triplicate and a blank determination was run as every tenth determination.

The digesting mixtures were as follows:

Kjeldahl mixture — 20 cc. acid, 10 gm. K_2SO_4, .7 gm. yellow HgO.

Gunning mixture — 20 cc. acid, 10 gm. Na_2SO_4, .2 gm. copper sulphate.

Kansas City Protein Referee Board mixture — 20 cc. acid, 7 gm. Na_2SO_4, .5 gm. $HgSO_4$, and .1 gm. $CuSO_4$.

With the one-gram sample, 20 cc. of acid was used throughout for the reasons given in the previous section.

Referring now to Table IX, it was found at low heat that the Kjeldahl (8) method was the only one which digested the samples completely in less than one hour. With this intensity of heat, the Gunning (9) method was very inefficient. In fact, we did not obtain complete oxidation in two hours with this method. It took 75 minutes to digest the sample with the Kansas City Protein Referee Board (4) method, while only an hour was required with the Kjeldahl method.

TABLE IX

INFLUENCE OF TIME, METHOD, AND SAMPLE ON PROTEIN TEST RESULTS

Sample number	Kjeldahl method* Time, minutes 15	30	45	60	75	90	105	120
				High heat				
	%	%	%	%	%	%	%	%
12,004	12.77	12.79	12.85	12.86	12.88	12.88	12.88	12.88
12,006	15.28	15.33	15.43	15.45	15.39	15.40	15.40	15.40
12,008	11.23	11.37	11.40	11.41	11.40	11.40	11.42	11.43
				Medium heat				
12,004	12.65	12.71	12.77	12.88	12.87	12.87	12.87	12.87
12,006	15.10	15.22	15.28	15.43	15.42	15.43	15.44	15.42
12,008	11.17	11.29	11.39	11.39	11.39	11.39	11.40	11.40
				Low heat				
12,004	12.61	12.72	12.84	12.88	12.88	12.80	12.88	12.88
12,006	15.12	15.18	15.29	15.45	15.45	15.45	15.39	15.45
12,008	‡	11.29	11.34	11.40	11.42	11.44	11.43	11.40
Sample number	K. C. P. R. R. method† Time, minutes 15	30	45	60	75	90	105	120
				High heat				
	%	%	%	%	%	%	%	%
12,004	12.65	12.70	12.82	12.82	12.82	12.82	12.82	12.82
12,006	15.20	15.38	15.38	15.35	15.38	15.38	15.35	15.38
12,008	11.17	11.37	11.39	11.40	11.44	11.44	11.42	11.40
				Medium heat				
12,004	12.48	12.65	12.82	12.87	12.88	12.82	12.85	12.82
12,006	15.22	15.38	15.38	15.39	15.38	15.38	15.38	15.38
12,008	‡	11.29	11.34	11.46	11.41	11.42	11.44	11.40
				Low heat				
12,004	‡	12.48	12.54	12.77	12.85	12.82	12.82	12.82
12,006	‡	15.04	14.98	15.15	15.38	15.33	15.38	15.40
12,008	‡	11.19	11.23	11.29	11.44	11.40	11.40	11.43
Sample number	Gunning method* Time, minutes 15	30	45	60	75	90	105	120
				High heat				
	%	%	%	%	%	%	%	%
12,004	12.37	12.74	12.84	12.82	12.83	12.87	12.81	12.85
12,006	14.88	15.10	15.32	15.36	15.38	15.36	15.38	15.38
12,008	11.00	11.34	11.40	11.39	11.39	11.44	11.41	11.40
				Medium heat				
12,004	11.86	12.71	12.71	12.88	12.82	12.86	12.85	12.88
12,006	14.59	15.10	15.22	15.38	15.38	15.38	15.38	15.38
12,008	10.89	11.29	11.34	11.40	11.40	11.44	11.43	11.42
				Low heat				
12,004	‡	‡	12.48	12.54	12.48	12.48	12.71	12.77
12,006	‡	‡	14.65	15.03	15.16	15.16	15.22	15.22
12,008	‡	‡	11.23	11.06	11.17	11.23	11.28	11.34

*Association of Official Agricultural Chemists. †Kansas City Protein Referee Board.
‡Not clear.

At medium heat, the Gunning method becomes more satisfactory and is in line with the other two methods. The digestion time with the Kansas City Protein Referee Board method was reduced 20 per cent by raising the heat from low to medium, whereas the time was reduced 50 per cent when the Gunning method was used. By using high heat it was found that all three methods were on a par. It was further brought out when using high heat with a one-gram sample and 20 cc. of acid, that no longer than 45 minutes is necessary completely to oxidize the sample.

A summary of the investigations in this section shows that protein test results will vary, depending upon the length of time of digestion, and that this period of digestion depends upon the intensity of the heat. At high heat, by all three methods, complete oxidation can be obtained in 45 minutes; at medium heat, in 60 minutes. At low heat, the Kjeldahl method was the only one giving correct results in less than an hour. With the Kansas City Protein Referee Board method 75 minutes was necessary. The Gunning method was not satisfactory at this intensity of heat.

TABLE X

PROTEIN CONTENT OF 19 SAMPLES OF WHEAT RUN BY THREE METHODS AT TWO DIFFERENT INTERVALS OF HEAT AND TIME

Sample No.	Series 1—High heat, 45 min. Kjeldahl method	K.C.P. R. B. method	Gunning method	Two hours Kjeldahl method
	Per cent	Per cent	Per cent	Per cent
1	11.86	11.91	11.91	11.86
2	12.88	12.94	12.88	12.88
3	10.20	10.26	10.26	10.20
4	11.00	11.06	11.06	11.06
5	13.11	13.17	13.17	13.11
6	12.31	12.37	12.37	12.37
7	13.22	13.17	13.11	13.08
8	13.22	13.24	13.22	13.24
9	7.92	7.86	7.92	7.87
10	12.82	12.82	12.82	12.82
11	10.54	10.54	10.54	10.54
12	8.26	8.26	8.21	8.25
13	9.92	9.92	9.92	9.97
14	12.82	12.82	12.82	12.82
15	15.30	15.30	15.36	15.38
16	11.41	11.43	11.40	11.40
17	10.77	10.72	10.66	10.66
18	12.88	12.88	12.88	12.82
19	15.36	15.40	15.40	15.43

Sample No.	Series 2—Medium heat, 60 min. Kjeldahl method	K.C.P. R. B. method	Gunning method	Two hours Kjeldahl method
1	11.91	11.91	11.91	11.86
2	12.88	12.88	12.88	12.88
3	10.20	10.20	10.26	10.20
4	11.11	11.06	11.06	11.06
5	13.18	13.11	13.11	13.11
6	12.37	12.25	12.31	12.37
7	13.11	13.11	13.11	13.08
8	13.24	13.22	13.21	13.24
9	7.87	7.98	7.87	7.87
10	12.88	12.77	12.82	12.82
11	10.60	10.54	10.60	10.54
12	8.26	8.21	8.21	8.25
13	9.97	9.97	9.92	9.97
14	12.81	12.82	12.83	12.82
15	15.33	15.39	15.33	15.38
16	11.41	11.41	11.41	11.40
17	10.66	10.66	10.72	10.66
18	12.88	12.88	12.88	12.82
19	15.40	15.45	15.40	15.43

In order to check further the times recorded above, 19 samples of wheat representing the five commercial classes of wheat were digested for two hours. The same samples were also digested for 45 minutes at high heat and for 60 minutes at medium heat. As we believe that low heat will never find place in commercial protein testing, it was not tried out. In the aggregate, with this intensity of heat the results are unsatisfactory. The oxidation is not regular and duplicative results are not the rule. The data from the check series, proving that 45 minutes is sufficient to oxidize a one-gram sample at high heat and that 60 minutes is sufficient to do the same at medium heat, are given in Table X.

However, these figures are given for very carefully controlled heat conditions. It was pointed out earlier that we are able to control the heat during the day and on different days by means of a gas pressure regulator, which is not the case with most laboratories. It is a common observation to note changes in flow of gas as more or less burners are used. This, of course, will change the flow of heat to each individual flask, depending on the number in operation and the pressure in the gas mains during the day. We feel, therefore, that in recommending 45 minutes at high heat, the variables mentioned will be eliminated, as in many cases almost complete oxidation was obtained at 30 minutes. The same is true for the data at medium heat, using 60 minutes.

Using two grams of sample, it will take 60 minutes to complete digestion at high heat and 75 minutes at medium heat. This is approximately 30% longer time than was necessary with a one-gram sample.

Influence of Size of Sample Ground on Protein Test Results

From the collaborative data, it was found that the amount ground varied from 6 to 150 grams. In order to determine how much effect the grinding of small and large amounts would have on the protein test results, 1000-gram portions of each of the 12 samples submitted for collaborative tests were mixed thoroly and passed through the Boerner (1) sampler and 8-, 15-, 30-, 60-, and 125-gram portions were drawn off, ground, and protein tests made.

The data given in part A of Table XI record the protein percentages without taking into consideration the moisture content of the sample. Differences of as much as 0.4 per cent of protein were obtained from the results of grinding varying quantities of wheat. The greatest differences were obtained with samples of 8 and 15 grams. This is due in some measure to a large loss of moisture from the samples during grinding. As much as 3% of moisture will be lost in the grinding process.

TABLE XI

INFLUENCE OF SIZE OF SAMPLE GROUND ON PROTEIN TEST RESULTS

Part A

(Results are on the basis of the moisture as present in sample)

Lab. No.	Size of sample ground					Maximum difference Protein
	8 Gm. Protein	15 Gm. Protein	30 Gm. Protein	60 Gm. Protein	125 Gm. Protein	
	%	%	%	%	%	%
11,998	10.94	10.60	10.77	10.72	10.66	.34
11,999	12.08	11.86	11.86	11.86	11.86	.22
12,000	13.00	13.11	13.11	13.05	13.05	.11
12,001	12.94	12.82	12.82	12.77	12.75	.19
12,002	13.22	12.88	12.94	12.82	12.88	.40
12,003	15.39	15.28	15.28	15.33	15.10	.29
12,004	12.37	12.25	12.20	12.20	12.20	.17
12,005	13.05	12.77	12.84	12.82	12.77	.28
12,006	14.31	14.48	14.53	14.53	14.53	.22
12,007	10.15	10.20	10.20	10.15	10.20	.05
12,008	10.66	10.66	10.60	10.54	10.60	.12
12,009	11.11	11.11	11.06	11.06	11.06	.05

Part B

(Results are on the basis of 13.5% moisture)

Lab. No.	Size of sample ground					Maximum difference Protein
	8 Gm. Protein	15 Gm. Protein	30 Gm. Protein	60 Gm. Protein	125 Gm. Protein	
	%	%	%	%	%	%
11,998	10.55	10.35	10.50	10.48	10.50	.20
11,999	11.69	11.69	11.61	11.58	11.65	.11
12,000	12.61	12.66	12.67	12.63	12.62	.06
12,001	12.51	12.50	12.51	12.42	12.44	.09
12,002	12.60	12.46	12.52	12.58	12.46	.14
12,003	14.71	14.76	14.82	14.89	14.79	.18
12,004	11.81	11.71	11.75	11.79	11.83	.12
12,005	12.45	12.35	12.39	12.44	12.44	.09
12,006	13.96	14.13	14.25	14.21	14.21	.29
12,007	9.62	9.83	9.89	9.79	9.83	.27
12,008	10.25	10.35	10.31	10.29	10.38	.13
12,009	10.90	10.80	10.76	10.78	10.78	.14

From the data in part B of Table XI it can be seen that there are some differences in the protein test results due to size of sample ground, even when the moisture factor has been considered. The smaller the size of the sample ground, the larger are these differences. That is to say, grinding only 8 to 15 grams of sample introduces an appreciable error even if moisture conditions are controlled. The differences in protein results when 30 grams or more are ground are very small, being in most cases well within the usual limit of error, 0.1 per cent. It is believed, however, that these grindings were carried out much more carefully than is the case in the average protein laboratory. The usual practice is to fill a small paper container directly from the grain sack with little or no cleaning. It is quite

possible that with wheat which contains a considerable amount of foreign material or dockage such practices will lead to erroneous results. It is common knowledge that the dark, hard, and vitreous kernels of wheat in any given sample, have more protein than the soft and chalky kernels. Differences in protein content will average 1.5% and cases are on record in which the differences were as great as 4.0% between the protein content of the dark, hard, vitreous kernels, and the soft and chalky kernels. If the amounts of these types of kernels vary appreciably, differences in results are bound to occur. This has been brought out by Frank (3) and is supported by the data in Table XII.

TABLE XII

PROTEIN CONTENT OF YELLOW AND CHALKY AND DARK HARD AND VITREOUS PORTIONS OF HARD RED WINTER AND HARD RED SPRING WHEATS

	Hard red winter wheats		Hard red spring wheats	
	Dark, hard, and vitreous	Chalky	Dark, hard, and vitreous	Chalky
Number of samples.....	156	156	70	70
Average protein content, per cent	12.54	11.11	13.15	11.42
Maximum " " " "	16.24	15.21	16.24	14.31
Minimum " " " "	8.82	7.40	11.58	9.74
Range in " " " "	7.42	7.81	4.66	4.57

Therefore, one can not be too careful in getting a representative sample of the grain to be analyzed before making the tests.

The method of cleaning the sample for analysis is not uniform. Sieves, dockage testers, and forceps are used to remove foreign material. Cases are known in which the small sized high protein kernels of "prescription wheat" were regularly sifted out, resulting in low protein tests. Such practices only lead to confusion.

At present, most laboratories are equipped with small burr flouring mills. These give very satisfactory grinding service. The only precaution that can be pointed out is to be sure they are cleaned thoroly before additional samples are run through. This can be done by running through not less than 15 to 20 grams of the new sample before taking any for test.

In Table XIII the investigations made on this point are recorded. A sample of high protein wheat was ground until no further meal was obtained and the residue was left in the mill. A second sample of low protein wheat, in 5-, 10-, 15-, 20-, and 25-gram portions, was then passed through and the protein test made on each grinding. After the 25-gram portion was run through, the mill was cleaned and a second 25-gram portion of the same sample was ground and protein tests made. This low protein sample was cleaned with 5-, 10-, 15-, 20-,

and 25-gram portions of the high protein wheat. The experiment was repeated with a second high and low protein wheat.

It is evident that from 15 to 20 grams of wheat are sufficient to clear the mill for any new samples. A smaller amount will cause erroneous results.

TABLE XIII

GRAMS OF WHEAT NECESSARY TO CLEAN AN ARCADE GRINDER IN ORDER TO OBTAIN CORRECT RESULTS

Treatment	Protein
Series I	
	Per cent
Sample 12,003,	15.45
" " cleaned with 5 gm. 11,998	12.25
" " " " 10 gm. "	10.94
" " " " 15 gm. "	10.72
" " " " 20 gm. "	10.72
" " " " 25 gm. "	10.72
Series II	
Sample 11,998,	10.72
" " cleaned with 5 gm. 12,003	13.39
" " " " 10 gm. "	15.28
" " " " 15 gm. "	15.45
" " " " 20 gm. "	15.50
" " " " 25 gm. "	15.45
Series III	
Sample 12,007,	10.26
" " cleaned with 5 gm. 12,006	13.22
" " " " 10 gm. "	14.36
" " " " 15 gm. "	14.45
" " " " 20 gm. "	14.48
" " " " 25 gm. "	14.50
Series IV	
Sample 12,006,	14.50
" " cleaned with 5 gm. 12,607	11.80
" " " " 10 gm. "	10.32
" " " " 15 gm. "	10.20
" " " " 20 gm. "	10.25
" " " " 25 gm. "	10.29

Influence of Fineness of Division of Sample on Protein Test Results

The fact that very little difficulty is experienced in getting concordant results when making protein determinations on flour is evidence that fineness of division may have a bearing on the accuracy of protein tests in wheat.

In order to study this point, 100-gram portions of the same twelve samples of wheat before described were analyzed in five different states of division, ranging from the whole seed to that condition where 100% of the sample passed through a No. 36 grits gauze. The fineness of division series in detail were: whole kernel, kernel ground so that 15, 30, 60, and 100%, respectively, passed through a No. 36 grits gauze. Before analysis, the samples were carefully mixed on paper to insure uniformity.

The data on this subject are given in Table XIV. Part A records the analyses in which no consideration was given to the moisture content, and part B the data on a 13.5% moisture basis.

It is very evident that the fineness to which a sample is ground influences the accuracy of protein tests. This is doubly true when moisture is not considered. The maximum difference under such conditions, as is shown in Table XIV, part A, was found to be 0.45 per cent. The results with whole kernels are irregular. Sometimes they are high and at other times low, as compared with the results from carefully ground samples.

TABLE XIV

INFLUENCE OF FINENESS OF DIVISION OF SAMPLE ON PROTEIN TEST RESULTS

Part A

(Results are on the basis of the moisture as present in sample)

Lab. No.	99% passing 36 G. G.	60% passing 36 G. G.	30% passing 36 G. G.	15% passing 36 G. G.	Whole seed	Maximum difference
	%	%	%	%	%	%
11,998	10.60	10.77	10.66	10.52	10.32	.45
11,999	11.91	11.91	11.80	11.66	11.63	.28
12,000	12.94	13.11	12.88	12.88	13.05	.23
12,001	12.78	12.82	12.60	12.60	12.54	.28
12,002	12.75	12.71	12.60	12.60	12.37	.38
12,003	15.45	15.28	15.16	15.28	15.16	.29
12,004	12.20	12.14	12.08	12.03	12.08	.17
12,005	12.75	12.77	12.77	12.65	12.77	.12
12,006	14.53	14.53	14.25	14.14	14.08	.45
12,007	10.22	10.15	10.15	10.15	10.09	.13
12,008	10.60	10.72	10.54	10.49	10.43	.29
12,009	10.94	11.06	11.00	10.83	10.66	.40

Part B

(Results are on the basis of 13.5% moisture)

Lab. No.	99% passing 36 G. G.	60% passing 36 G. G.	30% passing 36 G. G.	15% passing 36 G. G.	Whole seed	Maximum difference
	%	%	%	%	%	%
11,998	10.47	10.53	10.51	10.52	10.26	.27
11,999	11.62	11.65	11.67	11.66	11.59	.08
12,000	12.62	12.71	12.58	12.59	12.80	.22
12,001	12.45	12.51	12.40	12.43	12.38	.13
12,002	12.42	12.41	12.39	12.40	12.23	.19
12,003	14.85	14.78	14.72	14.87	14.81	.13
12,004	11.75	11.80	11.70	11.75	11.77	.10
12,005	12.34	12.45	12.46	12.50	12.55	.21
12,006	14.25	14.20	14.27	14.28	14.02	.26
12,007	9.84	9.77	9.83	9.88	9.85	.11
12,008	10.38	10.41	10.37	10.35	10.31	.10
12,009	10.81	10.83	10.80	10.73	10.56	.27

Eliminating whole seed, it is surprising to find that one can obtain such concordant results as are shown here, regardless of the fineness of division of the sample. This is attributed to the care taken in

mixing the sample after grinding, as preliminary trials on mill-run material gave very irregular results. Altho nothing of significance developed (the data from whole kernel excepted) from the standpoint of fineness of division, the experiment brings out the point that if ground samples are thoroly mixed after grinding, reliable analyses will result, even tho the samples have not been ground to a fine state of division. It has been our experience and observation that unless samples are thoroly mixed, incorrect results will occur. Moreover, grinding to a fine state of division eliminates the necessity for extensive mixing.

Influence of Moisture Content of Sample on Protein Test Results

When samples are ground in an attrition mill in preparation for digesting, changes in moisture are bound to occur. How great is this error depends upon such factors as size of sample ground, moistness of sample, how cool the mill remains with continued use, etc.

Experience in this laboratory has been that the smaller the sample ground, the larger the moisture loss. In the experiment listed in Table XIV, sample 11,998 contained 10.34% moisture when 8 grams were ground, and 11.41, 11.26, 11.56, and 12.20% when 15, 30, 60, and 125 grams were ground. Sample 12,002 contained 9.25, 10,29, 10.63, 11.82, and 12.34% when the same amounts were ground; and sample 12,005 had 9.52, 10.73, 10.35, 10.85, and 11.42% under the same conditions.

Naturally such changes in moisture content will influence the protein results, as has been pointed out by Shollenberger and Coleman (12) in Miscellaneous Circular No. 28 of the Department of Agriculture.

Moist or wet wheat is not only difficult to grind but will lose a greater percentage of moisture than dry wheat. Questions have been frequently asked as to how great this loss is and how the protein results would differ if they were reported on a moisture test, such as the Brown-Duvel (1) test: also how other results would vary from this if different methods of making moisture tests were used.

To obtain data on this point, samples of wet wheat were obtained and moisture determinations made by the following methods: Brown-Duvel (1); whole kernels heated for 120 hours in the water oven at a temperature of 99.5° C.; the same kernels ground and placed in the vacuum oven for five hours at 99.5° C. and four inches of mercury pressure; for five hours at 108° C. in an electric oven; and for five hours at 99.5° C. in a water jacketed oven. Twenty samples were tested.

By the Brown-Duvel (1) method, the average moisture content of the samples was 14.9%. Drying whole seed to constant weight in the water oven gave an average of 15.01% moisture. By the vacuuum oven method, the moistre content was 14.17%; by the electric oven method, 13.90%; and by the water oven method, 13.68%. The maximum difference by all methods was 1.39%. Some of this, of course, can be attributed to loss of moisture due to grinding. Necessarily, if a uniform method is not used for making moisture tests, correct protein results cannot be obtained.

The data in Table XV illustrate this point further and show the necessity for recognizing moisture as a factor in making protein tests. The data here show a range of 1.46% in the moisture content of the samples submitted, enough difference to change the protein content of a sample of wheat, especially one of high protein content, appreciably.

Distillation Technic

Investigations show that the outstanding factors necessary to secure reliable results from the distillation side of protein testing are: use of traps of sufficient size to prevent spray from being carried over during distillation, more frequent "blank" determinations, use of standard solutions of sufficient strength to be carefully measured and to enable the analyst to work efficiently, use of sufficient receiving acid to catch all the ammonia formed from samples having a large range in protein content, use of sufficient reagent to precipitate mercury when this is used as a catalyst, and accurate preparation of standard solutions.

The experiences of this laboratory have been an exact duplication of the findings of Paul and Berry (10) on the subject of Kjeldahl traps, and we need but emphasize the necessity of using large double-lipped traps, whenever possible, to secure the best results, especially when the distillation is carried on rapidly. The use of traps of various sizes and shapes on the same still will lead to erroneous results.

Blank determinations should be run more frequently. Blanks are run by some of the collaborators only every week or every 3 or 4 days. Blanks run on the same reagents for several successive days have been known to vary from 0.1 to 0.2 cc. of 0.1N alkali. This in terms of protein is equal to 0.06 to 0.12%.

As for receiving acids, it is believed that the use of half normal solutions should be eliminated as their use introduces too great a chance for error. For convenience, especially when using a two-gram sample, 0.2 normal acid has given satisfaction. Solutions for back titrations should not be stronger than 0.1253 normal, preferably 0.1 normal.

TABLE XV

MOISTURE RESULTS OBTAINED BY VARIOUS LABORATORIES ON TWO SERIES OF WHEAT SAMPLES SUBMITTED IN SEALED TIN CONTAINERS

Laboratory Identification	1 to 40	41 to 80	81 to 120	121 to 160	161 to 200	201 to 240	241 to 260	261 to 280	281 to 300	301 to 330	331 to 356	357 to 385
	%	%	%	%	%	%	%	%	%	%	%	%
1	13.04	13.28	11.51	12.33	12.27	10.49	11.04	11.45	13.35	10.23	13.78	12.70
2	12.75	13.10	11.40	12.20	11.95	10.10	11.02	11.41	13.17	10.13	13.57	12.54
3	13.34	13.77	11.86	12.75	12.56	10.58	.....	.....	.....	.....	.....	.....
4	.....	.....	.....	.....	.....	.....	11.58	11.74	13.81	10.58	14.12	13.07
5	13.64	13.97	12.14	12.97	12.78	10.81	.....	.....	.....	.....	.....	.....
7	.....	.....	.....	.....	.....	.....	11.26	10.85	12.66	10.37	12.96	12.03
*16	12.37	12.73	11.10	11.85	11.45	9.88	9.94	10.40	12.40	9.43	12.98	12.01
28	.....	.....	.....	.....	.....	.....	10.18	10.66	12.44	9.50	12.66	11.80
37	13.05	13.20	11.41	12.26	12.13	10.29	10.95	11.15	13.50	10.50	13.82	12.65
Total	78.19	80.05	69.42	74.36	73.14	62.15	75.97	77.66	91.33	70.74	93.89	86.80
Average	13.03	13.34	11.57	12.39	12.19	10.36	10.85	11.09	13.05	10.11	13.41	12.40
Maximum	13.64	13.97	12.14	12.97	12.78	10.81	11.58	11.74	13.81	10.58	14.12	13.07
Minimum	12.37	12.73	11.10	11.85	11.45	9.88	9.94	10.40	12.40	9.43	12.66	11.80
Range	1.27	1.24	1.04	1.12	1.33	.93	1.64	1.34	1.41	1.15	1.46	1.27

*It is not certain whether these results are on the basis of the samples submitted in sealed cans.

As the usual wheat crop will have a protein content varying from 8 to 20% (7), it is necessary to have present sufficient acid to hold 35 milligrams of nitrogen if a one-gram sample is digested, or 70 milligrams if a two-gram sample is digested. These conditions would require 12.5 cc. of 0.2 normal acid when a one-gram sample is digested, and 25 cc. when two-gram samples are taken for analysis.

Again referring to the article by Paul and Berry (10) we find first, that their figures for the volume of distillation are correct, namely, practically all the ammonia passes over with 75 cc. of the distillate and all of it with 100 cc.; and, second, that when sufficient acid is present to neutralize 85% of the distillate, no loss of the uncombined ammonia takes place. It is desirable to have all the ammonia combined, however. We further find it advisable to use 50 cc. of water in the receiving flask so that the nose of the glass tubing will be immersed.

As far as receiving acids are concerned, there is no difference between hydrochloric and sulphuric. It was thought by some that hydrochloric acid would not hold the ammonia distilled over at the high temperatures sometimes occurring in the distillation flasks. This is not the case, as can be seen by consulting Table XVI.

TABLE XVI

INFLUENCE OF TEMPERATURE ON ABILITY OF HCL, H_2SO_4, AND BORIC ACIDS TO HOLD AMMONIA

Acid	Temperature, ° C.							
	25	30	35	40	45	50	60	70
	Per cent NH_3 retained							
HCL	100	100	100	100	100	100	100	100
H_2SO_4	100	100	100	100	100	100	100	100
Boric	100	100	100	99.25	98.44	96.71	90.19*	86.89

*Can smell NH_3 at this temperature.

This table records the data comparing the ability of HCL, H_2SO_4, and boric acid to hold ammonia at increased temperatures. For this study, 10 cc. of approximately 0.1 normal NH_4OH was added to 0.1 normal HCl and H_2SO_4, and 4% boric acid solutions. The acid was present to the extent of 50% more than was necessary to neutralize the ammonia. The temperature varied in 5-degree intervals, from 25° C. to 50° C., then by 10-degree intervals to 70° C. It will be seen that there was no loss of ammonia when hydrochloric or sulphuric acid was used, but that boric acid began to lose ammonia at 40° C. and the amount lost gradually increased as the temperature was raised to 70° C.

Boric acid will also release, or lose, ammonia on standing. Titrations were made after adding ammonia and leaving stand for 1, 2, 3, 4, 5, 6, 7, 8, 16, and 24 hours. No ammonia was lost in 4 hours

but at the end of 5 hours, 0.85% of the original ammonia was lost and at 6, 7, 8, 16, and 24 hours, 0.86, 1.23, 1.99, 2.89, and 5.18% of ammonia was lost at the intervals listed. Boric acid, therefore, can be used with safety only when the temperature of the distillate is not greater than 35° C. It must also be titrated within 4 hours after the ammonia has been introduced. It is not safe to allow it to stand over night, contrary to the findings of Scales and Harrison (11).

Mercury Precipitants

The use of potassium and sodium sulphide seems to be general among collaborators using mercury as a catalytic agent. But four of the collaborators used sodium thiosulphate for this purpose. This last reagent, providing it is as effective as the first two, has a decided advantage over the sulphides as it is less expensive and has no repulsive odor. Tests were made of its efficiency as a mercury precipitant and it was found that one gram of sodium thiosulphate is sufficient to precipitate nine-tenths of a gram of metallic mercury. It was also found during the investigation that this amount of mercury will hold back approximately 13% of the nitrogen present. This is close to the figures as given by Paul and Berry (10). It is therefore suggested that this reagent be given larger use, especially where objection is made to the odor of the sulphides of potassium and sodium, as is often the case in office buildings.

Standard Solutions

In order to gather some information relative to the accuracy of standard solutions, eight ounces of sulphuric acid were sent to each collaborator with the request that he standardize the solution and let us know its value. These figures are given in Table XVII and show that the normality as submitted by 34 collaborators varied from 0.13003 to 0.1256, or a difference of 0.00443. Expressed in terms of protein, this is equivalent to 0.35 per cent, a very appreciable amount when it is considered that the Kansas City Board of Trade requires a re-check analysis when the protein test varies more than 0.12 of one per cent. We can not help but feel, moreover, that special attention has been given to this standardization work and that the usual routine in this field would show even wider variables.

As an added interest, the same sample of acid was sent to the United States Bureau of Standards for test and their normality value is given under the caption X in Table XVII. This value was obtained gravimetrically by weighing the sulphate as barium sulphate. This value was further checked volumetrically by titration with a standard

alkali solution. The two values were identical. No non-volatile sulphates were found.

TABLE XVII

NORMALITY FIGURES GIVEN TO A SAMPLE OF SULPHURIC ACID BY 34 COLLABORATORS

No. of collaborator	Normality submitted	No. of collaborator	Normality submitted
1	.1276	18	.1280
2	1286	20	.1268
3	.1290	21	.1274
4	.1268	22	.12735
5	.1297	23	.1277
6	.1276	24	.1288
7	.1290	27	.1282
8	.1285	29	.1286
9	.1286	31	.13033
10	.1274	33	.12771
11	.1276	34	.12826
12	.1282	36	.1288
13	.1288	37	.1268
14	.12775	39	.1295
15	.12852	42	.1276
16	.1294	45	.1256
17	.1296	X*	.12974
	Average normality found,		.12823
	Maximum " "		.13003
	Minimum " "		.12560
	Range in " "		.00443
	Protein equivalent		.35%

*Bureau of Standards.

The results obtained by 34 collaborators show that a decided effort should be made to standardize the manner of determining the values of normal solutions. Eight distinct reagents were used to determine the normality of alkali solutions, namely, benzoic acid, constant boiling point hydrochloric acid, hydrochloric and sulphuric acid solutions of known normality, oxalic acid, potassium-acid—phthalate, potassium bitartrate, and succinic acid. In many cases the protein results are calculated direct from the values of the standard alkali. In other instances, however, a cross titration is made and the results are calculated on the value of the standard acid. Such a practice usually results in lower normality values, especially when compared with results obtained by precipitation and weighing of either hydrochloric or sulphuric acid.

In this connection it is suggested that careful consideration be given to standardizing solutions with potassium acid phthalate. This salt can now be obtained in very pure form, is non-hygroscopic, and can be very accurately weighed on account of its high molecular weight. Furthermore, the end point in titration with sodium or potassium hydroxide is remarkably clean cut. The use of this salt as a standard

in volumetric analysis has been discussed fully by Hendrixson (5, 6) and by Dodge (2), and their data are well worth studying. The use of potassium acid phthalate has been entirely satisfactory in this laboratory.

From the preceding discussion it is evident that careful attention will have to be given to the points brought out in the text in order to secure correct results when making protein tests.

The practice of buying on a protein test is becoming so universal that appeals and disputes from test results will tend to increase in number unless some uniform procedure is adopted for sampling and analyzing the samples.

It is believed that the present practice of sampling (1) as used by the various grain inspection departments is satisfactory if carefully carried out. Having obtained the sample, however, the matter of keeping its identity can not be over-emphasized.

If this point is not heeded, it is of little use to make any further tests. Practices that lead to the drying of the grain; the taking of samples for tests after they have been on the trading floor all day subject to drying, handling, and contamination; the placing of moist wheat in paper sacks preparatory to grinding, will never lead to correct results and understanding of the protein test.

As the protein test is usually given in connection with the numerical grade of the sample of wheat, samples for analysis should be cleaned in the same manner as for the numerical grade, not by the methods used by many laboratories.

In making protein tests, it appears that speed, efficiency, and inexpensiveness are the most desired essentials. To attain speed, an efficient source of heat is absolutely necessary. Lacking this, speed can not be obtained, as catalytic agents offer only a limited degree of help and aid digestion to a limited extent.

The three methods studied in this report are equally efficient, provided heat conditions are optimum. Reference to the text will show where they differ with fluctuations in heat intensity.

From an expense standpoint, provided heat conditions are optimum, the Gunning method can perhaps be used more economically than either of the others, other conditions being equal.

If reductions in the amounts of catalytic agents are made from the figures given in the formulae studied, they will have to be compensated for by more intense or longer heat.

It was found that there was no difference between electricity and gas as sources of heat, electricity having the advantage of giving greater intensity of heat under the conditions in this laboratory.

To be of service to the grain trade, laboratories operated by some neutral agency should be established at the more important grain markets. These laboratories would be of great value in helping other types of laboratories to standardize their solutions, methods, technic, and so forth. Eventually, this will have to be done.

Summary

No uniform method is used by different laboratories for determining the protein content of wheat. Three general types of methods, with various modifications, were used by 38 different laboratories. With these methods the 38 laboratories reported a maximum difference of 3.15% of protein. Investigations were planned to study the reasons for these differences.

Twenty cc. of concentrated sulphuric acid are necessary to digest and retain all the ammonia liberated from a one-gram sample of wheat at the intensity of heat usually employed by most laboratories. Twenty-five cc. are necessary when a two-gram sample is used.

Heat is the most important factor in digesting wheat samples, as it controls the time a sample should be digested.

Yellow and red oxides of mercury are equivalent in all quantities as catalytic agents, of which 0.5 gram gives satisfactory results.

Mercuric sulphate gives slightly lower results than the other two salts studied.

Sodium sulphate and potassium sulphate are equal as catalytic agents. Ten grams of either gives best results when 20 cc. of acid is used. Sodium sulphate, however, has the disadvantage of forming a salt cake with the usual amounts of sulphuric acid used for digestion. This salt cake can be prevented by using a mixture of 60% K_2SO_4 and 40% Na_2SO_4.

Copper sulphate as a catalytic agent is of little value at low intensities of heat.

A two-gram sample gives slightly higher results than a one-gram sample. From an error standpoint, the advantage of using a two-gram sample is not great enough to warrant its use.

Considering the extra time needed to digest a two-gram sample, the two-gram sample is undesirable.

The Kjeldahl method is the only method with which wheat samples can be completely oxidized in less than an hour at all intensities of heat. The Gunning method and the Kansas City Protein Referee Board Methods are not satisfactory at low heats.

No difference in results can be attributed to methods if the heat is sufficiently intense. Wheat can be completely oxidized in 45 minutes

at high heat and in 60 minutes at medium heat, when a one-gram sample is used. It takes 30 per cent more time to digest a two-gram sample.

The heat intensities used were as follows:

Low heat—50 cc. water evaporated from 200 cc. in 20 minutes.
Medium heat—100 cc. water evaporated from 200 cc. in 20 minutes.
High heat—150 cc. water evaporated from 200 cc. in 20 minutes.
The time was recorded when the water began to boil.

Not less than 30 grams of wheat should be ground for protein tests to secure results representative of the bulk sample. Errors of as much as 0.3 per cent will result if smaller samples are used.

Wheat should be ground whenever possible. Results from whole seed are irregular when compared to data obtained from carefully ground seed.

Great care should be taken to retain the moisture in the sample, as changes in moisture induce changes in the protein test results. Differences of 0.6 per cent have been noted in these investigations.

In the distillation process careful attention should be given to the use of traps and the making of blank determinations.

At least 100 cc. of distillate should be collected in acid which will hold the equivalent of 35 to 70 milligrams of nitrogen, depending upon whether a one- or two-gram sample is used.

There is no choice between sulphuric and hydrochloric as receiving acids.

Boric acid will lose ammonia at 40° C., the loss increasing with the rise in temperature.

Boric acid solution containing ammonia must be titrated within four hours in order to incur no losses of ammonia.

Sodium thiosulphate is an acceptable substitute for sodium or potassium sulphide.

Careful attention also should be given to standardizing normal solutions. The error in making such standards is much too large. Errors in terms of protein equal 0.35 per cent.

The use of potassium acid phthalate as a standard solution is recommended.

A referee laboratory should be established by some neutral agency to settle disputes of any nature, arising out of the practice of making protein tests.

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THE RESEARCH ASSOCIATION OF BRITISH FLOUR MILLERS

By E. A. FISHER

The Research Association of British Flour-Millers, Sopwell, St. Albans, England

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Before the war the competition of imported flour had been overcome by the British flour-milling industry to the extent that approximately 90 per cent of the material requirements were met by home manufacture. At the end of the Great War, British flour millers were much exercised by the necessity which faced the industry of regaining and surpassing this position. It was realized that the industry must avail itself of all possible means of improving the efficiency of its processes and the quality of its products and that research along these lines was desirable and essential. In such work individual effort, while indispensable to individual success and prosperity, is not enough. It is possible for only the very largest firms to undertake continuous research into many problems the solution of which leads to improved processes or reduced costs. Only by co-operative effort can smaller firms carry out investigations on any adequate scale or provide facilities for inquiry into technical questions, both of general and of particular interest. It was for these reasons that The Research Association of British Flour Millers was incorporated in September, 1923. It was regarded as essential that such a research association should have as part of its equipment a mill running on commercial lines, in which the results of experimental work could be checked and developed upon a practical commercial scale. The research association is supported partly by annual subscriptions from its members and partly by the Government, through the Department of Scientific and Industrial Research. Should the membership and consequently the income from subscriptions increase, the government grant will also increase on a Pound for Pound basis. By the terms of the government grant none of this income can be used directly on a concern run on ordinary commercial lines for profit, such as a commercial flour mill; so it has been found necessary to establish two separate organizations which are financially distinct:

(1) The research association, which is in the form of a company not trading for profit, limited by guarantee; and

(2) The demonstration mill, which is organized as an ordinary limited liability company under the name of the The British Flour Mills, Ltd.

On grounds of general accessibility, both for visitors to the mill, for purposes of reference, and for availability of supplies and distribution of products, it was considered that the mill should be situated in the neighborhood of London, and it was eventually decided to take over the New Barnes Mill, Sopwell, St. Albans. This mill has a capacity of five to six sacks (7 to 8½ barrels) of flour per hour and is being brought up to date by the replacement of much of the existing machinery by new and larger machines many of which have been presented to the mill by the manufacturers. The control of the mill is in the same hands as the control of the research association, the board of directors of the milling company being, with the exception of one member, identical with the executive committee of the research association. This is essential in order to insure co-operation and co-ordination of effort between the scientific and the commercial organizations. Moreover, as the efficiency of the whole scheme depends upon the mutual support of the research association and the milling company, it was decided that all members of the research association should be shareholders in the milling company and that, as a condition of their membership in the research association, they should be required to subscribe for shares in the milling company in proportion to the capacities of their respective mills. Further, power to hold shares in the milling company is restricted to members of the research association.

The senior scientific and technical staff consists at present of a director of research, and three chemists, one of whom is in charge of the analytical and advisory work and another of the library and information bureau. There is in addition an assistant analyst, and a baker will be appointed as soon as the experimental bakehouse has been built and equipped. Provision is also being made in the new laboratories for a biologist. A suitable site near the mill has been acquired and the necessary laboratories, administrative offices, and bakery will be erected on it. Preliminary plans have been drawn up and it is hoped that these buildings will be ready for occupation in the summer or autumn of 1925. Meanwhile, a vacant block of laboratories some miles from the mill has been rented for one year and work has already been begun.

The new buildings will contain three laboratories (chemical, analytical, and biological), directors' room, library, office, workshop, a constant temperature and humidity chamber, and ample storage accommodation; and the site is sufficient in area to admit of considerable extension to the buildings as future requirements may necessitate.

An experimental bakehouse is to be erected as a separate building on the laboratory site, and proving cabinets and ovens will be installed for baking normal 2-pound loaves and small half-pound loaves.

In addition to the bakery equipment, a small model mill is to be installed. Milling operations will frequently be necessary on small quantities, especially in connection with the laboratory conditioning of wheat and with investigation of new varieties when small quantities only may be available. The working of such a mill will have to be carefully compared with and if possible standardized against the working of the commercial mill. The value of such a small mill, however, is wider than is indicated by the necessity of milling small laboratory samples of wheat. There are many problems in flour-milling that are difficult to attack under commercial conditions in a full-sized mill. The temperature and humidity of the atmosphere for example—both of which vary greatly from day to day or even from hour to hour, as all night shifts are not uncommon in flour mills—have important effects on the milling processes as well as on the products. The study of "manufactured weather," that is, the study of temperature and humidity control in connection with flour milling has not made great strides in England altho such control is the rule in the great textile trades. It is a difficult and costly matter to experiment along such lines in a commercial mill, but such working conditions can be easily controlled and varied with a model mill. The research association's model mill will be housed in a room in which the temperature and humidity can be varied at will or automatically kept constant at any desired combination.

The general aims of the research association are indicated by the following citations from the first report of the director of research to the council at their meeting held on March 12, 1924.

> The work can, broadly speaking, be divided into three branches, viz., the library or information bureau, the analytical and advisory work, and the pure research work.
>
> The library will contain ultimately, it is hoped, all books, pamphlets, journals, both scientific and technical, in English, German, and French that bear directly on the milling industry in its wider sense; publications concerning the raw materials and finished products and by-products of the industry are even more important than those dealing with the technical processes and will be included. In addition a large mass of material dealing with cereals and cereal products is to be found widely scattered in many scientific journals in other libraries; the numerous bulletin publications of the U. S. A. Government, and of the various state colleges and experiment stations are examples, while the chemical and biochemical literature dealing with starches and proteins is enormous. The greater part of this has to be read, abstracted, collated, and card-indexed. This

collating of literature is a branch of scientific activity which is not always appreciated at its true value. The importance of such an information bureau as this to a band of scientific workers is very great indeed, and besides this, it is hoped from time to time to issue for the information of members, bulletins based on the published literature, on various problems of the industry, such, e.g., as conditioning or bleaching. These bulletins will not, of course, be written in scientific language for scientific workers, but in plain non-technical English; they will be meant for millers and not for chemists.

This work has been considered of such importance that it has been deputed as a full-time engagement to a member of the scientific staff specially appointed for the purpose.

The second branch of our activities is the analytical or advisory work. It is not intended that the research association shall undertake ordinary routine analyses for its members. But it is hoped that when millers are up against difficulties or complaints respecting the quality of their products they will turn to their research association for help. In such cases samples will be analyzed, and when necessary baking or other tests carried out and reports based upon such tests issued to the members concerned.

The section of our work dealing with pure fundamental research will be a thing of slower growth. . . .

In this branch of our activities one can say, as a kind of first approximation, that most of our problems are concerned with the question of *quality* in wheat and flour. Conditioning is perhaps next to quality in the miller's mind, and altho it is undoubtedly a long way behind quality in importance, it has important connections with it.

Now quality is really a huge complex of problems each of which is in its turn complex. We can, for example, distinguish at least three *groups* of factors that contribute to weakness in flour: (a) there may be an insufficient amount of perfectly good gluten; (b) gluten may be adequate in quantity but poor in quality; or (c) there may be a satisfactory amount of perfectly good gluten, but factors such as those connected with gas production may be deficient.

A really comprehensive attack on this problem "can be made only by the united efforts of the chemist, physicist, biochemist, plant and animal physiologist and geneticist (and practical miller and baker), working in close collaboration. . . ."

It is obviously impossible that all these branches of science should be represented on the (research association) staff—the financial problem alone would be too big—but I am hopeful of being able to work in collaboration with workers at other research institutes.

These are the three main branches of our activities, but they are not to be regarded as in any sense water-tight . . . compartments. Such a hard and fast division is alien to the spirit of scientific investigation, and can only be tolerated—for it can never be really justified—in very large institutions. The three branches I have described will work in close association—each will be absolutely vital to the work, I might almost say the existence, of the other two, and the only separation between them will be the merely physical one of a few inches of bricks and plaster. If an

> integrating factor were needed at all, such a factor exists in the demonstration mill. The mill is there for our use, and, even more important, for our inspiration; it will serve as a reminder of our common aims and objects, of our unity of outlook and of effort, and will later, I hope, provide us facilities for developing yet a fourth branch of our activities—that of chemical engineering, the study of the technical processes for converting wheat into flour. This, however, is a matter for the future, and can not be dealt with at this early stage of our history.

These ideas were elaborated and extended into a paper on "The Field for Research in the Flour Milling Industry" which the director of research read at the convention of the national association, at Folkestone, in June, 1924. This paper was printed in extenso in "Milling" for June 21 (1924), pp. 708A-711, and in "The Miller" for July 7 (1924), pp. 404-410.

Following on this general survey of the whole field of research, a special research program has been drawn up by the director of research, i. e., a detailed list of special problems and groups of problems to be attacked in the research association's laboratories, experimental bakehouse, and mill. This program, which has been accepted as the official research program of the association by the council of the association, is given below:

It is impossible if only on account of the strictly limited resources of the research association to carry out investigations in every branch of the industry. Research will be begun along a few promising lines and as the work progresses and as the research and technical staff become more and more familiar with the details of milling processes and with the general working of the demonstration mill, fresh problems will present themselves and their full significance will be appreciated. In suggesting fresh problems for investigation, the advisory work carried out for members by the research staff will be of great value.

The following scheme comprises the main lines of work contemplated. The sections indicated by an asterisk are those on which attention will first be concentrated.

*I. A study of the flour stream of New Barnes mill.

*II. A comprehensive study of two wheats, one strong, e. g., No. 1 Northern Manitoba; and one weak, e. g., an average sample of English, excluding Yeoman, and of flours and flour blends obtained from them. The following eleven series of flours will be studied: 100 per cent No. 1 Northern Manitoba, and mixtures of No. 1 Northern Manitoba with 10, 20, 30, 40, 50, 60, 70, 80, and 90 per cent average English, and 100 per cent average English.

A thoro study of these wheats and flours will be made in the laboratory, in the experimental mill, and in the bakehouse and these will serve as *reference wheats and flours* with which others will be compared.

IIA. The effect of storage under a variety of conditions on both wheats and flours.

*IIB. Conditioning of the two reference wheats:

(1) A study of rates of water absorption by, and of rates of drying and other moisture relations of single berries will be made.

(2) Samples of the two wheats will be conditioned in a special conditioning oven at various temperatures from 80° F. upwards at 10° intervals and at various relative humidities from 30% upwards at 10% intervals.

(3) Effect of damp on wheat and flour:

(a) cf. (2) above.

(b) Effect of prolonged exposure to damp at temperatures sufficiently low to avoid heating.

(c) Effect of repeated wetting and drying (of grain and flour) of wheat in stook exposed to frequent showers.

III. Wheat and flour survey of the country.

A. It is hoped to take up the work of the home grown wheat committee by working in close connection with other institutions interested in wheat growing and wheat breeding. In this connection a study will be made of

(a) new varieties

(b) effect of environmental conditions on different varieties, especially with a view to

(c) finding best varieties suited to local conditions.

B. Besides growing the most suitable wheat locally, each local miller wants to mill the most suitable flour; moreover the best flour in one district is not necessarily the best in another, hence a comprehensive study of flour standards and flour grades will be made, e. g., samples of a miller's best flour will be compared

(a) with other good samples from other districts,

(b) with inferior samples from the same and other mills.

This really resolves itself into a study and comparison of all the various grades of flour produced by our member's mills, i. e., ultimately a survey of the flour grades of the country.

*IV. The study of certain physico-chemical and colloid properties, especially viscosity and hydrogen-ion concentration of flour-in-water suspensions, doughs, and breads.

*V. The study of improvers and their effects on baking quality and other properties of flours. (This partially comes under IV.)

VI. Flour bleaching:

(a) bleaching proper

(b) indirect effects of bleaching, e. g., effect of Cl on H-ion concentration and hence on quality, or the effect of bleaching on enzymic activity of flour.

VII. Physiology of the wheat berry.

A. Study of the progressive development of the wheat berry with special reference to the colloid phenomena involved. Out of this arise more particularly

(a) varietal differences and effects of environment on these

(b) effects of drought
(c) effects of frost
(d) effects of sprouting
(e) effects of tempering
} on colloid and other properties of the endosperm and hence on baking quality of the flour.

B. Respiration of wheat in relation to commercial storage and ageing.

VIII. Chemical, physical, and biological study of the changes occurring during doughing and baking. Under this heading comes study of yeasts and the enzymes associated with them, especially diastase, zymase, the proteases, and phytase. The latter two have been but little investigated and their possible rôle in doughing and baking is obscure.

IX. The staling of bread, especially in relation to flour quality.

X. Physical and physico-chemical study of the actual processes of converting wheat into flour; the physics of milling processes, e. g., the study of mill conditioning (as distinct from wheat conditioning), of crushing and grinding, and of sifting by sieves and air currents.

XI. Wheat offals, especially with a view to standardization and grading.

*XII. Information bureau; all literature bearing on milling problems to be abstracted, collated, and card-indexed. In this connection summaries of literature on various points will be issued at intervals.

BOOK REVIEW

Colloid Symposium Monograph. Vol. II. Papers presented at the second national symposium on colloid chemistry, Northwestern University, June, 1924. Edited by Harry N. Holmes, chairman, committee on the chemistry of colloids, National Research Council. The Chemical Catalog Company, New York, 1925. VII + 368 pp. Price $5 net.

This monograph presents the twenty papers presented at the Second National Colloid Symposium. They cover a wide field and are of interest to every colloid chemist. The topics cover almost every field of colloid chemistry—clays, emulsions, soil, proteins, yeast, plasticity, and the orientation of molecules. The papers are all well written and most of them contain much original experimental data. It is a book that should be in the library of every chemist interested in colloids.

The papers which are of special interest to cereal chemists are:

Plasticity in colloid control, by Eugene C. Bingham, pp. 106-113. The advantages of plasticity methods for identification of substances is especially emphasized. As compared with the errors of many per cent in technical viscometry, fluidity is capable of very precise measurement, about one-tenth of a per cent, particularly in relative measurements. Some examples listed in which the properties of flow, i.e., plasticity, are important are starch, cellulose, soaps, fats and greases, chocolate, condensed milk, gelatin, glue, gluten, and flour.

Polar emulsifying agents, by Harry N. Holmes and H. A. Williams, pp. 135-137. The early works of Langmuir and of Hardy on the definite orientation of molecules at the surfaces of liquids are reviewed. A report of the authors' work on classes of polar emulsifying agents not commonly supposed to be colloidally aggregated in the liquids used is given. Among these agents studied were alcohols, acids, esters, aldehydes, ketones, and nitriles. "The proteins and soaps are polar and in addition their molecules form large colloidal aggregates, hence the greater strength of their films in emulsions. There is really a great difference in the emulsifying powers of the soaps and of the lower alcohols, but the important point is that certain substances in true solution do form distinct emulsion films."

The orientation of molecules in the surface of liquids, by William D. Harkins, pp. 141-173. This is a very interesting article describing the work of the writer on the orientation theory of surface structure. Some of the "short statements concerning the fundamental principles of surface theory, together with statements of a few important facts" are:

"The molecules in the surfaces of liquids seems to be oriented, and in such a way that the least active or least polar groups are oriented towards the vapor phase."

"If the solvent is polar, such as water, then solutes will in general be positively adsorbed in the surface if they are less polar than water, and the least polar end of the molecule will be turned outward. Solutes more polar than water are negatively adsorbed."

"The stability of emulsoid particles seems to be brought about by orientation of molecules at the interface with the medium of dispersion."

The effect of ammonium salts upon the swelling of colloids and upon the growth of yeast at various temperatures, by Ellis I. Fulmer, pp. 204-208. It was found that the maximal concentration effect of ammonium salts on the growth of yeast was about 0.0353 N at 30° for the four ammonium salts, chloride, sulfate, nitrate, and tartrate. If, however, the temperature is raised to 40°, a higher concentration of salt is necessary to obtain the best growth. The name "thermal buffer" is suggested for the salt and indicates the importance of the composition of the medium upon the thermal death point of an organism or the efficiency of heat in sterilization. It was also noted that the optimal concentration of the ammonium salt for the growth of yeast for each temperature tested was identical with that in which a protein (wheat gluten) was least swollen. Equations are given for calculating the normalities of ammonium chloride required for the maximal growth of yeast and the minimal swelling of gluten at any temperature.

Physico-chemical studies on proteins I. The prolamines, their composition in relation to acid and alkali binding, by Walter F. Hoffman and Ross Aiken Gortner, pp. 209-368. The contents of this article are brought out in the following from the summary and conclusions.

The known alcohol soluble proteins from wheat, spelt, rye, oats, barley, corn, and kafir, and the unknown alcohol soluble proteins from durum, emmer, einkorn, teosinte, and sorghum, as well as casein and fibrin, were prepared and analyzed. The data on the elementary analysis did not show any striking differences between the various proteins.

The nitrogen distribution, the free amino nitrogen, the free carboxyl groups, the true ammonia nitrogen, and the tryptophane and cystine content of this series of proteins, were studied. A close similarity was noted between the proteins prepared from different types of cereals. Altho certain differences in chemical analyses were found between the proteins prepared from a single type of cereals, these differences were not sufficiently marked to enable one sharply to divide the cereal prolamines into sub-classes.

Chemical characteristics of the alcohol soluble proteins of the cereals place them in the same divisions as do their biological reactions. These chemical data, however, do not sub-divide the individuals of the wheat group into the three groups, i.e., spelt, emmer and einkorn, as do the studies on crosses, chromosome number, susceptibility to rust, etc. Teosinte and *zea mays* have also been shown to be very closely related genetically. These studies, together with other experiments,[1] show that the prolamines from the two groups differ widely.

The binding of acid and alkali by the various proteins was studied. Because of its recognized accuracy the potentiometric method was used almost exclusively. All the proteins, regardless of their chemical composition, bound, gram for gram, approximately the same amount of acid or alkali, when the final hydrogen-ion concentration was greater than pH 2.5 and the hydroxyl-ion concentration was greater than pH 10.5. The logarithms of the equivalent of acid or alkali bound by the proteins when plotted as ordinates against the logarithms of the equivalents of acid or alkali added, or the final pH as abscissa, gave straight lines. The constants a and b for the formula for a straight line were calculated for all such curves resulting from the experiments with the present series of 14 proteins. The values for the constants are almost identical for the proteins prepared from the same type of cereals.

Approximately equivalent amounts of hydrochloric, sulfuric, and phosphoric (molar) acid were bound by a unit amount of protein, when the acids are compared on the normality basis.

A negative temperature coefficient was obtained when the experiments on the binding of hydrochloric acid and sodium hydroxide were carried out at 15°, 25°, and 35° C. and when the final hydrogen-ion concentration was more than pH 2.5 and the hydroxyl-ion concentration was more than pH 10.5.

The analytical data in regard to the amino acid content of the prolamines are not sufficiently accurate to enable final conclusions to be drawn as to the chemical groups responsible for the chemical bind-

[1] Since this paper went to press the work of Lewis, Wells, Hoffman, and Gortner on "An immunological and chemical study of the alcohol-soluble proteins of cereals" (Proc. Soc. Exp. Biol. Med. Vol. 22, pp. 185-1924) appeared. The proteins of the "wheat group" were compared with proteins of the corn group and with each other by means of four immunological methods. "The prolamines from emmer, einkorn, spelt, and durum are closely related to gliadin and glutenin from *T. vulgare* while those from teosinte and kafir are closely related to zein from *Zea mays*. The former are more closely related to gliadin than they are to glutenin, while the protein from teosinte is more closely related to zein than is kafirin. No reactions were obtained between antisera for the corn group with proteins from the wheat group or conversely." This again shows the close genetic relationship of the different groups of cereals.

ing of alkali. In the case of acid binding, however, a correlation of $r=0.9923\pm0.00275$ was found between the free amino nitrogen of the protein as determined in the Van Slyke apparatus and the equivalents of acid bound at pH 2.8; and a correlation of 0.9918 ± 0.00312 was obtained between the sum of the free amino nitrogen plus one fourth of the arginine nitrogen (the free amino group of the guanidine nucleus) and the equivalents of acid bound at pH 2.5.

The measured isoelectric "point" of a protein probably is not a definite point but should in all probability be referred to as an "isoelectric range." The position of this isoelectric range on the pH scale is dependent on the chemical composition of the protein. The calculated isoelectric point is very near the hydrogen-ion concentration of neutral water. This is what would be predicted on the theory that at the higher concentrations of acid and alkali, the binding of acid and alkali follow the adsorption law. The calculated isoelectric points are not related to the chemical composition of the protein.

The other papers are:

General principles of ion effects on colloids, by Leonor Michaelis. pp. 1-15.

The electro-viscous effect in rubber sols, by G. S. Whitby and R. S. Jane. pp. 16-28.

Determination of particle size, by W. J. Kelly. pp. 29-36.

An improved method of sedimentary analysis applied to photographic emulsions, by F. F. Renwick and V. B. Sease. pp. 37-45.

Sols with non-spherical particles, by Herbert Freundlich. pp. 46-56.

Studies with the Kinoultramicroscope, by E. O. Kraemer. pp. 57-69.

A new method for the determination of the distribution of size of particles in emulsions, by Alfred J. Stamm. pp. 70-79.

Properties of clays, by A. V. Bleiminger. pp. 80-98.

Bentonite, by Jerome Alexander. pp. 99-105.

The theory of adsorption and soil gels, by Neil E. Gordon. pp. 114-125.

The rôle of colloids in soil moisture, by George John Bouyconcos. pp. 126-134.

Iodine as an emulsifying agent, by Harry N. Holmes and H. A. Williams. pp. 138-140.

The supercentrifuge, by Lee H. Clark. pp. 174-184.

The effect of surface energy on colloidal equilibrium, by H. O. Halvorson and R. G. Green. pp. 185-194.

Bacteria as colloids, by Arthur I. Kendall. pp. 195-203.

W. F. HOFFMAN.

SUSTAINING MEMBERS OF AMERICAN ASSOCIATION OF CEREAL CHEMISTS

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Gooch Milling & Elevator Co., Lincoln, Neb.
W. W. Hatton, c/o Messrs. Sale and Frazer, Tokio, Japan
Hecker-Jones-Jewell Milling Co., New York City, N. Y.
The Fleischmann Company, New York.
The Hoffman Mills, Enterprise, Kan.
The Hungarian Flour Mills Co., Denver, Colo.
The Ismert-Hincke Milling Company, Kansas City, Mo.
International Milling Co., Minneapolis, Minn.
Kansas Milling Co., Wichita, Kan.
Larabee Flour Mills Corporation, Kansas City, Mo.
Liberty Yeast Corporation, New York City.
Milton-Hersey Co., Ltd., Winnipeg, Canada.
Minnesota State Experimental Flour Mill, Minneapolis, Minn.
Montana Experiment Station Grain Laboratory, Bozeman, Mont.
V. H. Noury & Van der Lande (Novadel Processes), Buffalo, N. Y.
Thomas Page Milling Company, Topeka, Kan.
Pillsbury Flour Mills Company, Minneapolis, Minn.
Geo. P. Plant Milling Co., St. Louis, Mo.
Practicum. Ltd., London, England
Purity Baking Co., St. Paul, Minn.
Shellabarger Mill & Elevator Company, Salina, Kan.
Societe Anonyme de Minoteries et D'Elevateurs A Grains, 24 Rue Royale, Bruxelles, Belgium
Southwestern Milling Co., Kansas City, Mo.
Sperry Flour Company, Portland, Ore.
Bernard Stern & Sons, Inc., Milwaukee, Wis.
Wallace & Tiernan Co., Newark, N. J.
Washburn-Crosby Company, Minneapolis, Minn.
Western Canada Flour Mills Co., Winnipeg, Canada
Wichita Mill & Elevator Co., Wichita Falls, Tex.
Wichita Flour Mills Co., Wichita, Kan.